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(MSc THESIS)

DEVELOPMENT OF A NEW SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF HYDROGEN PEROXIDE

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ÖZET

HİDROJEN PEROKSİT TAYİNİ İÇİN YENİ BİR SPEKTROFOTOMETRİK YÖNTEMİN GELİŞTİRİLMESİ

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Bu tez gerçek su örneklerinde hidrojen peroksit tayini için spektrofotometrik bir yöntem önermektedir. Bu yöntem hidrojen peroksitin demir(II)-EDTA çözeltisiyle alkali ortamda kompleksleştirilmesine dayanmaktadır.525nm’de maksimum absorbansa sahip olan mor renkli demir(III)-EDTA-perokso kompleksi oluşturularak ölçüm alınmaktadır. Stabilizatör madde ve derişimi, Fe(III), EDTA ve amonyak derişimi gibi çeşitli parametreler 525nm’de sırasıyla; $S_2O_3^{2-}$ 0.05 mol/L, 0.003 mol/L ve 0.05 mol/L ve 5mol/L olarak tespit edildi. Kimyasal reaktiflerin eklenme sırası ve analiz süresi de ayrıca optimize edilmiştir.

Mor renkli demir(III)-EDTA-peroxo kompleksi oluşumuna dayanan hidrojen peroksit tayin yöntemi 267 L mol^{-1} cm^{-1}molar soğurma katsayısına sahiptir ve $5.0x10^{-6}$-$4.08x10^{-3}$ mol/L aralığında Beer yasasına uyum göstermektedir.Sandell’s duyarlığı 0.188 ug/cm^2 dir.Belirtme sınırı ve saptama sınırı değerleri sırasıyla, $2.5x10^{-6}$ ve $8.5x10^{6}$ mol/L’dir. $2.0x10^{4}$ mol/L H_2O_2 derişiminde gün içi ve günler arası tekrarlanabilirlik değerleri sırasıyla %1.5 ve %6.1 olarak hesaplanmıştır.

Yöntemde ayrıca su örneklerinde yaygın olarak görülen girişimci iyonların etkileri de incelenmiştir.Demir(II) haricinde neredeyse hiçbir iyon yöntemde girişimci etki göstermemiştir,dolayısıyla yöntem ekstra bir örnek hazırlama basamağı gerektirmeden doğal su örnekleri için doğrudan uygulanabilmektedir.

Bu tez çalışması kapsamında önerilen yöntem sırasıyla içme suyu,çeşme suyu ve deniz suyu örneklerine uygulanmış ve %90 ve %118 arasında değişen kabul edilebilir geri kazanım değerleri elde edilmiştir.

Anahtar sözcükler:hidrojen peroksit tayini, Demir(III) perokso kompleksi, spektrofotometri, su analizi.

ABSTRACT

DETERMINATION OF A NEW SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF HYDROGEN PEROXIDE

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Supervisor: Assoc. Prof. Dr. Levent PELİT

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This thesis proposes a spectrophotometric method for the determination of hydrogen peroxide in real water samples. The method is based on the complexation of hydrogen peroxide with the Fe(III)-EDTA complex in the alkaline medium. A purple color of low stable peroxo-iron(III)-EDTA complex was formed, with maximum absorbance at 525 nm. Variety of parameters such as type of stylizer reagent and concentration, Fe(III), EDTA $S_2O_3^{2-}$ and NH_3 concentration were optimized as 0.003 mol/L, 0.05 mol/L 0.05 mol/L and 5 mol/L respectively at 525 nm. Reagent addition order and measurement time were also optimized.

Determination of hydrogen peroxide method based on the formation of the purpled color of peroxo Fe(III)-EDTA complex was obeyed to Beer's law in the range 5.0 x10^{-6} - 4.08x10^{-3}mol/L, with a molar absorption coefficient (at 525 nm) of 267.36 L mol^{-1} cm^{-1}.Sandel's sensitivity of the proposed method was also calculated as 0.188ug/cm^2. Limit of detection and limit of quantification was found as 2.5x10^{-6} and 8.5x10^{-6}mol/L. Intraday and interday relative standard deviation of the proposed method for 2.0x10^{-4}mol/L of H_2O_2 were found as 1.5% and 6.1% respectively.

The effect of interfering ions that is common in real water samples were also studied. Nearly none of common ions except Fe(II) showed interfering effect to proposed method so, the method can be easily acceptable to real water samples without any sample preparation step.

The proposed method was successfully applied to real water samples namely drinking water, tap water and seawater with acceptable recovery value between 90% and 118%.

Keywords: determination of hydrogen peroxide, iron(III) peroxo complex, spectrophotometry, water analysis

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ABBREVIATIONS

HRP	Horseradish peroxidase
DPD	N,N-diethyl-p-phenylenediamine
DMP	2,9-diemethyl-1,10-phenanthroline
AOP	Advanced Oxidation Processes
POHPAA	p-hydroxyphenylacetic acid
Luminol	5-Amino-2,3-hihydro 1,4phathlazinedione
Peroxo complex	H_2O_2-Fe(III)-EDTA complex
Scopoletin	7hydroxy6methoxy-2H-1benzopyran2one
S	sandell’s sensitivity
Es	specific extinction coefficient
Y	concentration of the substance in mg/L
LOD	Limit of detection
LOQ	Limit of quantification

1.INTRODUCTION

1.1 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a colorless, odorless liquid. Pure H_2O_2 is pale blue, unstable and has explosion risk so;it is generally stored in water solution.The boiling point of H_2O_2has been known as 150.2°C. Practically, H_2O_2 will exposed to potentially eruptive thermal degradation if it is heated to this temperature (Brauer,1963).

Hydrogen bonding forms between water and H_2O_2molecules so physical properties of aqueous solutions H_2O_2such as freezing point etc. quite differs from the pure water and H_2O_2. Melting point of pure water and pure H_2O_2are0°C and −0.43°C respectively. On the other hand melting point of 50%(w/w) solution of the H_2O_2 solution is −51°C. Similarly, the boiling point of the 50%(w/w) solution of the H_2O_2 mixtureis at 114°C. (Hydrogen Peroxide Technical Library, 2016).

The molecular structure of H_2O_2 molecule shows a nonlinear rotation with (twisted) C_2 symmetry. (Hunt,1965).Gaseous and solid phase molecular structures of H_2O_2 are considerably distinctive. The effects of hydrogen bonding discovers this properties in aqueous solutions and this properties is not observed in gaseous phase (Dougherty,2005).As can be seen from the Figure 1.1 solid state of H_2O_2 are tetragonal shape (Abrahams et.al,2005).

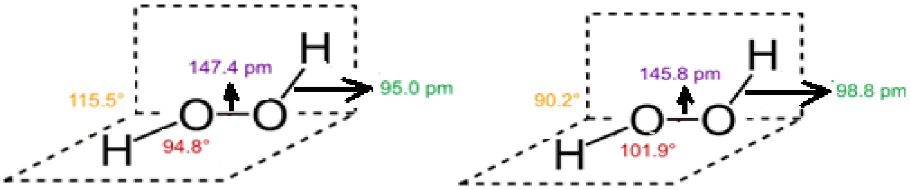

Figure 1.1. The gaseous and solid state molecular structure of H_2O_2(modified from Dougherty,2005)

H_2O_2 has a similar molecular structure analogues with O, N or S atom and form $H_m{-}X{-}X{-}H_n$ bonding arrangements with these atoms (X = O, N, S).All of these analogues shows similar asymmetric structures and they are thermodynamically unstable (Douglass, 1992; Thacker, 2010).

H_2O_2 is thermodynamically unstable and degrades to form oxygen and water according to following reaction.The degradation speed of H_2O_2 is increases at high pH, temperature and concentrated solution of it. The stability of H_2O_2 increases both increasing pH and decreasing temperature. Degradation of $H_2O_2$is catalyzed by several surface, such as iron,silver and platinum etc(Petrucci ed.2007).

$2H_2O_2 \rightleftarrows 2H_2O+O_2$

Degradation of H_2O_2 can also observed in the presence of transition metal ions, such asTi^{3+} or $Fe^{2+}$to form free radicals such as (HO·) and (HOO·).The enzymes can also catalyze the decomposition of H_2O_2 in biological medium. Oxygen and heat were produced by the decomposition of H_2O_2 and can cause a fire at high-concentration.

The oxidation and reduction properties of H_2O_2 depends on the pH of the medium. H_2O_2 is a powerful oxidizer reagent and the oxidation strength stronger than Cl_2 (chlorine), ClO_2(chlorine dioxide), and $KMnO_4$ (potassium permanganate) in acidic solutions.On the other hand, H_2O_2can be turn intohydroxyl radicals (·OH) that are highly reactive to catalysis of organic compound (Barbusıńskı, 2009).

1.2 The Usage of Hydrogen Peroxide

Many of foods packaged in cartons, tubes, bottles and foilsasepticallyclean by H_2O_2. These storage-stable products sustain the required shelf life and high product quality. To create a sterile environment, in the aseptic packaging units various treatment approaches for sterilization of materials and internal machine surfaces are used. For aseptic packaging equipment, sterilizing agents should effectively provide the same degree of protection in terms of microbiological safety, whichtraditional sterilization systems provide. For aseptic packaging H_2O_2 is the most common compound used as a sterilizing agent (Ansari, 2009).

At the same time H_2O_2 is an economical and powerful oxidizing agent due to its low molecular weight. That has found many uses in the chemical industry for the manufacture of organic compounds. H_2O_2uses in cosmetics and personal care products as an antimicrobial agent.In addition, it is one of the primary bleaching components as well as carbamide peroxide in tooth whitening products like pastes or gels (Shepherd, 2007).Peroxide based organic compounds such as acetone peroxide has been used for production of explosive materials(Block, 1991).On the other hand, in the presence of sorbent H_2O_2 itself also shows explosive properties. Peroxide is also used as a rocket fuel in the monopropellant (Hill, 2001; Wernimont, 2006).

H_2O_2 is generally used in wastewater treatment to eliminate organic impurities from water (Munter, 2001). This is obtained via advanced oxidation processes, is also called as the Fenton reaction (Falagas et. al., 2011). The detailed information Fenton reaction was given at part 1.5.

1.3 The Health Effects of Hydrogen Peroxide

H_2O_2 plays a physiological role as an a defense agent and oxidative stress marker in biological system (Alamiryet.al, 2008; Lippertet.al,2011; Yuanet.al,2012). Because of the damage of several classes of essential proteins by H_2O_2 in human body can cause various diseases such as cardiovascular, cancer, diabetes and neurodegenerative disorders (Miller,et.al, 2010; Paulsen,2009; Rhee,2006;Winterbourn,2008).

Reaction of H_2O_2 with catalase produces oxygen in the tissues and water because of decomposition of H_2O_2. When the amount of oxygen evolved exceeds the maximum solubility in blood the systemic venous and intravascular oxygen embolism effect occurs. When the dilute solutions of H_2O_2 ingested to human body some health effects observe such asgastric distension and emesis, gastrointestinal irritation, gastrointestinal erosions or embolism.

Concentrated solution of H_2O_2 shows more serious effect to body such as rapid loss of consciousness followed by respiratory arrest. Upper airway irritation, shortness of breath, hoarseness, inflammation of the nose and sensation of burning or tightness in the chest can be observed by intake of gas phase H_2O_2 by inhaled air. Exposure to high concentrations of H_2O_2 by air way can result in bronchi and delayed accumulation of fluid and severe mucosal congestion of the trachea in the lungs (Canadian Centre for Occupational Health and Safety, 1998).

The IARC (International Agency for Research on Cancer) has noted that there is insufficient data for the carcinogenicity of $H_2O_2$to humans. The overall conclusion of IARC was that $H_2O_2$is not classified as carcinogenic compound to humans but some mutagenic properties has been observed in *in vitro* systems. There is still a controversial on the cancer maker properties of H_2O_2.

1.4. Determination Methods for Hydrogen Peroxide

Hydrogen peroxide can be detected by four-analysis technique. These methods include; volumetric titration, spectrophotometric, fluorescence and chemiluminescence tecniques.

1.4.1.Titration methods

These methods can classified as iodometric, permanganate, and ceric sulfate methods.

Iodometric method is based on iodine formation in the presence of excess I^- at at acidic conditions according to reaction blow. The reaction is carried out in the presence of molybdate catalyst(Scott ,1939).

$$H_2O_2 + 2KI + H_2SO_4 \leftrightarrow I_2 + K_2SO_4 + 2H_2O$$

The formed iodine is titrated with a $S_2O_3^{2-}$according to the following reaction. The starch indicator is used to detect the titration endpoint.

$$I_2 + 2Na_2S_2O_3 \leftrightarrow Na_2S_4O_6 + 2NaI$$

Because of the decrease of iodine concentration by the titration, the color of solution turns pale yellow near the endpoint. After adding starch to medium a deep blue color forms. Titration is carried out until the solution turns to colorless (Kieber and Helz, 1986). Because of the insoluble complexes between the starch and iodine the indicator should be added near the endpoint of the titration. In the iodine formation step the solution should be kept for 5 minutes in the dark to get the more precise results. This method is used for the standardization of stock H_2O_2 solution. Variety of residual metal ions such as copper, iron, chromium and nickel catalyzes the decomposition of H_2O_2 and gives negative errors (Gordon et al., 1992).

Permanganate Method, manganese (VII) is reduced to manganese (II) with H_2O_2 according to following reaction (Schumb et al., 1955, Masschelein et al., 1977, Klassem et al., 1994)

$$2KMnO_4 + 5\ H_2O_2 + 3H_2SO_4 \rightleftarrows K_2SO_4 + 2MnSO_4 + 8H_2O + 5O_2$$

The titration reaction take place between 5 mol of H_2O_2 to 2 mol of $KMnO_4$ in acidic medium. The endpoint of H_2O_2titration with permanganate is detected from the development of pinkness color of $KMnO_4$ after end point (Hochanadel, 1952).

Some inorganic and organic substances react with permanganate cause a positive error and analyst should take in account for residual organic compounds before analysis.

Ceric Sulfate Method includes quantifying H_2O_2 concentrations via titration against Cerium(IV) by using ($Ce[SO_4]_2$) form. In this reaction Cerium (IV) is reduced to cerium (III) via H_2O_2 in acidic medium. The temperature of titration medium should not exceed above 10°C. Ferroin is used as an indicator for this titration method. When the color turns orange-blue titration is ended (Solvay Chemical Inc., 2004a)

1.4.2 Spectrophotometric Methods

Spectrophotometric methods are classified for five main categories namely cobalt carbonate, iodometric, titanium, HRP and peroxo vanadium method.

Cobalt Carbonate Methodincludes the oxidation of cobalt(II) to cobalt(III) byH_2O_2 (US peroxide, 2015). When cobalt(II) is oxidized to Co(III) by H_2O_2 in the concentrated bicarbonate solution[$Co(CO_3)_3$]Co) complex forms. The complex seems as dense green and has three absorption bands at 635 nm,440 nm and 260 nm. The analysis wavelength is advised as 260nm(Masschelein et al., 1977; Gordon et al., 1992;Bader et al., 1988)

Iodometric Method includes oxidizing iodide to form iodine in the presence of molybdate catalyst.

$$H_2O_2 + 2I^- + 2H^+ \rightleftarrows I_2 + 2H_2O$$

Produced I_2 react with excess I^- to form water solubleI_3^- complex according to following reaction. The color of $I_3^-$is detected by spectrophotometrically (Klassem et al, 1994).

$I_2 + I^- \rightleftarrows I_3^-$

At nearly neutral pH, a colour which is yellow will occur at 351 nm.The limit of detection is 50 µg/L and dome oxidants such as chlorineand transition metals are thought to interfere with the method.

Titanium Methodis based on the reaction between$H_2O_2$and $K_2TiO(C_2O_4)_2$to forma peroxo titanium complex inthe acidic medium. The complex has yellowy color and maximum adsorption band of colored complex is at 400nm(Solvay Chemical Inc, 2004b; Sunder and Hempel, 1997; Karpelvel et.al., 1997; Price et al., 1994; Volk et al., 1993).

Horseradish Peroxidase Method, includes the use of horseradish peroxidase (HRP).which is a hemoprotein capable of catalyzing the oxidation of some substrates by H_2O_2 (Worthington Biochemical Corporation, 2008). The reaction between HRP and $H_2O_2$is selective enough and resistive to interfering species. The reaction is going along the line below;

$2H_2O_2$+ reduced-forms$\rightleftarrows$ $3H_2O$ + oxidation-forms

The reaction is highly selective to H_2O_2 so a great deal of peroxide determination methodscan be used by HRP. These determination methodsinvolve; oxidizing the chemiluminescent genres, destructing the fluorescent genres and forming a matter which is easily detected by spectrophotometric methods. (Andreae, 1955).The methods called as leuco crystal violet method, DPD method and copper–DMP Method.

1.4.3.Fluorescence Methods

HRP Method, base on catalyzing the oxidation of certain substrates via H_2O_2. Two fluorescence methods involving HRP discussed below called POHPAA method and scopoletin method.

POHPAA(p-hydroxyphenylacetic acid) Method, includes producing a dimer from POHPAA in order to make fluorochrome in the presence of peroxidase. The mechanism of this reaction is very complex (Miller and Kester, 1988). During the process, peroxidase is oxidized from +3 to the +5 state. Oxidized form of peroxidase is in return reduced form by POHPAA to form POHPAA radicals. After that, POHPAA radicals formed in the reaction before, dimerize to form a product, which has fluorescent properties. The dimer has emission at 400 nm and excited at 313 nm. Primary anions and cations which is present in natural water has no big effect on the method (Kok et al., 1986). It is immune to the nitrate ions as well. Nevertheless, oxidants which are in the chlorine and hyperchloride forms interferes in a positive way with the method (Schick et al., 1997). The other method based on the reacition between scopoletin (7-hydroxy-6-methoxy-2H-1-benzopyran-2-one) and H_2O_2 (Price et al., 1994).

1.4.4 Chemiluminescence Methods

5-Amino-2,3-hihydro-1,4-phathlazinedione (luminol) which is a chemical phosphor stimulates a reaction sequence when mixed with H_2O_2 in the existence of a catalystthe reactions end up with the release of photons from a luminal sub-product.

Especially, a multistage reaction process proceeds along these lines (Yamashiro et al., 2004).

- Firstly,peroxide degredates in OH• radicalsin the existence of a catalyst.

- Then the luminol anions react with OH• radicals in order to produce luminol radicals.

- The luminol radicals react with oxygen radicals, that are produced by the reaction among OH• radicals and peroxide, to form a hyproperoxide intermediate.

- The hyproperoxide intermediate decomposes in 3-aminophthalate in an excited level of energy. The released photons proceeds to the ground state.

- The photons that are emitted, are detected through a photomultiplier tube.

To promote this sequence of reactions, a pH of nearly 10 has to be sustained to assure the existence of luminol anions. Either copper(II) (Madsen and Kromis, 1984) or. cobalt(II) can catalyze the degredation of peroxide(Burdo and Seitz, 1975). However, for natural water samples it is exposed to positive interference.

1.5. The complex Formation Between H_2O_2 and Fe(III)

Different catalysts havebeen used for decomposition of H_2O_2 including Fe(II) and Fe(III) (Weiss,1935). The salt of Fe(II), $FeSO_4$, is also called as Fenton's reagent and used for decomposition of organic substrates by H_2O_2(Tachiev et.all,2000) Decomposition of H_2O_2 by Fe(II) and Fe(III) can be performed according to following reactions (Fenton reaction) (Leat and Gallard, 1999).

$Fe^{3+} + H_2O_2 \rightleftarrows Fe^{2+} +.\cdot HO_2 + H^+$

$Fe^{2+} + H_2O_2 \rightleftarrows Fe^{3+} + \bullet OH + OH^-$

This reaction can be used for the decomposition of organic compounds (Yaman and Gündüz, 2015).

The reaction between H_2O_2 and iron ions can form by adding a strong complex-forming agent, such as ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA), at neutral or basic pH range. Chelating reagent play a significant role in the reaction mechanism of H_2O_2 with iron(III) and in the presence of EDTA at gives a purple color with H_2O_2 at basic solution. The decomposition of H_2O_2 and oxidation of organic substrates including bound and free EDTA catalyst by purple colored Fe(III)–EDTA peroxy complex which wasfirst published in 1956(Cheng, 1956). The step for the peroxo iron(III) complex formation can be summarized as follows. First step is the dissociation of H_2O_2 in alkaline solution according to following reaction (Holm et all, 1996).

$H_2O_2 \rightleftarrows H^+ + HOO^-$

After that the formation of Fe^{3+}–EDTA–peroxo complex is performed according to following reactions (Sharmaa et all, 2004).Reaction mechanism of Fe(III)-EDTA-peroxo complex is also presented in Figure 1.2.

$[Fe(EDTA)H_2O]^- + H_2O_2 \rightleftarrows [Fe^{3+}(EDTA)O_2]^{3-} + 2H^+ + H_2O$

$[Fe(EDTA)H_2O]^{2-} + H_2O_2 \rightleftarrows [Fe^{3+}(EDTA)O_2]^{3-} + H^+ + H_2O$

$[Fe(EDTA)H_2O]^- + HO_2^- \rightleftarrows [Fe^{3+}(EDTA)O_2]^{3-} + H^+ + H_2O$

$[Fe(EDTA)H_2O]^- + HO_2^- \rightleftarrows [Fe^{3+}(EDTA)O_2]^{3-} + H_2O$

The summary of these reaction presented in Figure 1.2.

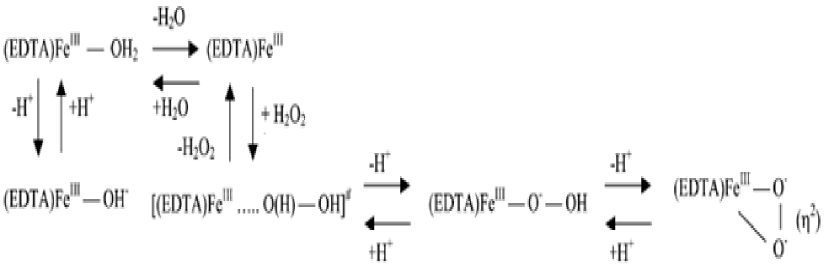

Figure 1.2 Reaction mechanism of Fe(III)-EDTA-peroxo complex(modified from Sharmaa et al, 2004).

Fe^{3+}-EDTA peroxocomplex have also catalyzed the organic substratesand polymerization of styrene(Brausam,2009).

There arevariety of studies on the complexion between H_2O_2 and Fe^{3+}-EDTA in aqueous environments. The chemical structure of H_2O_2 have been studied well (Figure 1.3) and the properties of peroxo complex was discovered by detailed spectrometric study. (Neese and Solomon, 1998; Cho, 2011).

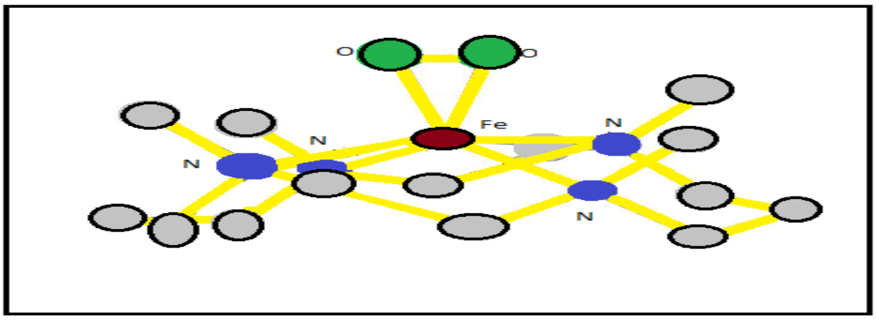

Figure 1.3. The structure of peroxo iron(III)-EDTA complex (modified from Cho, 2011)

1.6. Aim of the Thesis

Today, there is an increasing demand for the development of rapid analytical testing of clinical and environmentally important compounds determination at trace levels by an inexpensive and simple way. H_2O_2 is a reactive oxygen species, whichhas a great importance for chemistry, biochemistry and in the field of life sciences.H_2O_2, which is an unstable compound cause radical formation in some cases and mayhas carcinogenic effect to human. Because of the rapid degradation of H_2O_2, monitoring and identification of it with fast technique so important analytical problem.

Today advanced oxidation processes in water treatment business, 0_3 / H_2O_2, O_3 / UV and H_2O_2 / UV systems are used. There is trace levels of H_2O_2residual in water systems because of these reactions (Glaze, 1987). On the other hand, H_2O_2 can produce with both dark and photochemical reactions of organic compounds. Because of high evaporation of H_2O_2(Henry constant: 1.4 x 10^5mol dm^3 Atm^{-1}, 20°C) in the micromolar range helps resolve peroxide in water and allow the determination of this ppm (Finlayson, 1986). However, 0.1%, which is administered in the drinking water, are found to cause cancer in the mouse duodenum compositions 0.4% (Dresso, 2000).

Therefore, there need a simple, fast, accurate and reliable measurement method for determination of H_2O_2 in water samples. Many analytical methods in the literature; titrimetric, gravimetric, fluorimetric (Abbas, 2010), chemiluminescence (Shengmin, 2009), amperometric (Yifei,2005) and electrochemical sensors including enzymatic biosensors (Kozan, 2007), and liquid chromatography (Effkemann, 1998) was used for the spectrophotometric determination of H_2O_2. However, most do not have access to adequate and sensitivity of these are time consuming method. Therefore, in this thesis,a spectrophotometric method is aimed to determine H_2O_2 in a quickly, reliable and sensitive way for real water samples.

Purple colored Fe(III)-EDTA peroxo complex was used for the first time for the determination of H_2O_2 in the literature.

2.EXPERIMENTAL

2.1 Instrumentation

Spectrophotometric measurements were carried out using CARY 1OOBio UV-Visible spectrophotometer. Scan and kinetic mode were used throughout the spectrophotometric studies. Hellma analytics high precision quarts cells were employed for analyses.

2.2 Reagents

All reagents were of analytical reagent grade.Boric acid, sodium hydroxide(NaOH),copper sulphate ($Cu(SO_4)_2).3H_2O$), sodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$), bizmuth nitrate($Bi(NO_3))_3$, silver nitrate ($AgNO_3$), potassium iodide (KI), manganese(II) sulfate ($MnSO_4$), sodium oxalate ($Na_2C_2O_4$), sodium chloride (NaCl),sodium fluoride (NaF) ,mercury(II) chloride ($HgCl_2$), sodium sulfate (Na_2SO_3), nickel(II)nitrate ($NiNO_3$), potassium chloride (KCl) , potassium chromide (K_2CrO_4),4-buthylphenyl boronic acid,potassium iodate (KIO_3), ammonium iron(II)sulfate ($(NH_4)_2Fe(SO_4)_2$),stannic oxide (SnO_2), lead(II) nitrate ($Pb(NO_3)_2$), molybdenum dioxide (MoO_2),sodium thiosulfate ($Na_2S_2O_3$), sodium carbonate (Na_2CO_3), molybdenum trioxide (MoO_3), arsenic(II) oxide(As_2O_3), potassium bromide (KBr), zinc oxide (ZnO_2),calcium chloride ($CaCl_2$), and potassium dichromate ($K_2Cr_2O_7$) were used in experimental studies.

Solutions of EDTA were prepared from solid $Na_2(H_2EDTA)$ (Merck).$S_2O_3^{2-}$ solution were prepared from solid $Na_2S_2O_3$ (Kimetsan), Fe(III) solution were prepared from solid $FeCl_3.6H_2O$ (Merck), H_2O_2standard solutions were prepared by dilution of a 35 % (w/w) stock solution of H_2O_2 (Merck).Solutions of NH_3were used by taking from of 25 % (w/w) concentrated solution of NH_3 (Merck).

Stock standard solutions of $Na_2S_2O_3$ (0.1 mol/L) and H_2O_2 (0.1 mol/L) were freshly prepared by dissolving ultrapure water [Millipore Milli Q system (18.2 MΩ)]. Stock and standard solutions were prepared daily after standardization of H_2O_2 stock solution by iodometric method.

2.3Preparation and Standardization of Solutions

2.3.1 Preparation and standardization of $S_2O_3^{2-}$solution

0.1 mol/L of $S_2O_3^{2-}$ solution was prepared from $Na_2S_2O_3$ for the iodometric titration of H_2O_2 stock solutions. KIO_3 used as primer standard for standardization of $S_2O_3^{2-}$ solution by iodometric method. $I_2$is formed in the Erlenmeyer by reacting a standard (0.1mol/L) solution of $KIO_3$with KI in the acidic medium. After addition 1 mL of 0.1 mol/L KIO_3,1 mL 10% (%w/v) KI and 1 mL of 1 mol/L $H_2SO_4$was addedinto the Erlenmeyer. I_2 was produced from IO_3^- and I^- according to the following reaction.

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$$

The golden-brown colored iodine solution was titrated by using of $S_2O_3^{2-}$ solution. The $Na_2S_2O_3$ solution reacted with the liberated $I^-$and the color of the solution faded near the endpoint. After addition of a few drops of a freshly prepared starch to solution was added and a blue-black color observed. The titration was carried out until the solution becomes colorless. The titration reaction represented as following reaction.

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$$

2.3.2 Preparationand standardization of H_2O_2stock solution

The stock solution of the H_2O_2 was prepared daily after standardization of it. To prepare standard solutions of $H_2O_2$1 mL of concentrated H_2O_2 solution transferred to 100 mL volumetric flask and required dilution was made.

Freshly prepared stock solution of H_2O_2 standardized as follows. 5 mL of stock solution of H_2O_2 solution transferred to Erlenmeyer and added 5 mL of 1 mol/L H_2SO_4 to acidified the sample. Then 5 mL of 10% (%w/v) potassium iodide solution is added. Because of the slow reaction rate of iodide with H_2O_2, a few drops of 0.1 mol/L ammonium molybdate was added to solution for catalyzing by the reaction. The top of Erlenmeyer closed by parafilm and placed in dark medium for 5 minutes to complete the following reaction.

$$H_2O_2 + 2\ KI + H_2SO_4 \rightarrow I_2 + K_2SO_4 + 2H_2O$$

Finally, the generated I_2 was titrated with standard sodium thiosulfate solution according to following reaction: Starch solution was again used as indicator to detect the end point of titration.

I_2+ 2 $Na_2S_2O_3$→$Na_2S_4O_6$+ 2 NaI

2.3.3 Preparation of starch solutions

To prepare the starch solution firstly a 100 mL beaker cleaned,than ultrapure water transferred in it and boiled on an electrical heater. After that0.500 g of solid starch added to the boiled water and mixed for a few minutes. Finally, the solution was cooled and was filtrated before used.

2.3.4. Preparation of complexing reagent

Complexing reagent was prepared freshly before use. For this purpose, solid $FeCl_3$ transferred to a beaker and a few milliliters of ultrapure water added and mixed well. Then solid Na_2H_2EDTA added to this mixture and stirred until all compound dissolved. After that 10 mL of 25 %NH_3 stock solution added to mixture to get alkaline media. Finally, solid $Na_2S_2O_3$ added to the mixture and diluted to 25 mL in a volumetric flask.

2.4. Spectrophotometric Analysis Method of H_2O_2

Two different analysis step were used in H_2O_2 analysis. First way was formation of peroxo complex in the volumetric flask and then the measurement of spectrum after transfer of the peroxo complex to the quartz cell. Second way was the formation of the peroxo complex directly in the quartz cell and measurement of the absorbance with it. Because of low stability of the H_2O_2 best results were obtained with direct formation of the peroxo complex in the quartz cell. Measurement time of the direct preparation of peroxo complex it the cell and measurement it was at least two times faster than the first method. Therefore, peroxo complex were prepared in cuvette and directly measured with it at all experiments. The measurement method given as follows.

First of all the cell was treated with acetone and then was dried completely two remove residual water from the cell. After that, 2.0 mL of H_2O_2 containing solution was added to cell then 1.2 mL of concentrated NH_3 added to the medium. Finally 300 µL of complexing reagent (containing 0.5 mol/L EDTA, 0.03 mol/LFe(III), 5 mol/LNH_3) was added to final solution. Final concentration of the EDTA, Fe(III) and NH_3 in the cell were $5.0x10^{-2}$ mol/L, $3.0x10^{-3}$mol/L and 5.0 mol/L respectively. Purple coloredperoxo complex formed immediately and the colored solution was shacked well to get homogenous solution. The spectrophotometric measurementwas done as fast as possible after mixing the solution.

2.5. Sample Analysis Method

The water samples were filtrated by 0.25 µm PTFE filter to remove the particles from water samples before analysis. Then sample analysis was carried out according to part 2.4. After filtration, water samples except seawater directly analysed with proposed method. The sea water sample were 5 times diluted by pure water before analysis.

3. RESULT AND DISCUSSION

3.1. Absorption Spectra of Colored Peroxo Complex

Absorption spectra of aqueous solutions of Fe(III), Fe(III)-EDTA, Fe(III)-EDTA-NH_3 and colored complex of Fe(III)-EDTA-NH_3-H_2O_2 were recorded between 800-400 nm without baseline correction (Figure3.1). As can be seen from the Figure 3.1, a very sharp charge transfer band started around (500 nm)(Zena, 2013). After addition of EDTA into the Fe(III) solution sharp charge transfer band shifted tomore short wavelengths(450 nm) because of the complex formation of Fe(III) with EDTA. A similar absorption spectra was observed by the addition of NH_3 into the Fe(III)-EDTA solution. Except a small absorption band was observed at λ_{max}475 nm.When H_2O_2 was added to the Fe(III)-EDTA-NH_3 mixture a purple colored complex was observed immediately and a peak appeared at,λ_{max} 525 nm due to the formation of the Fe(III)-EDTA-H_2O_2 complex, as shown in Figure 3.1-d.

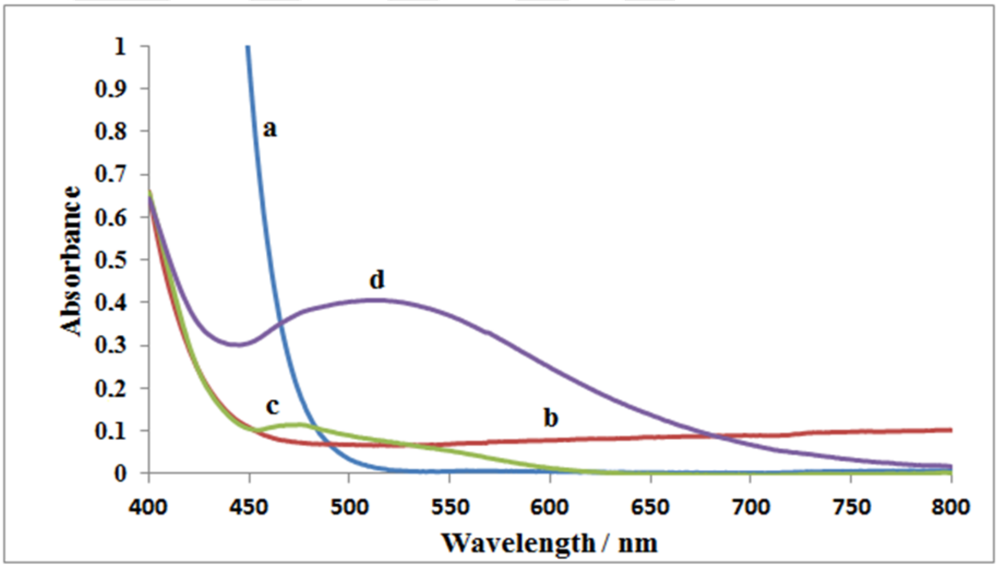

Figure 3.1. The spectra of a)Fe(III)solution, b) Fe(III)-EDTA solution,c) Fe(III)-EDTA-NH_3 solution and d) Fe(III)-EDTA-NH_3-H_2O_2

The color of Fe(III)-EDTA-NH_3 solution and its peroxo complex were also presented in Figure 3.2.

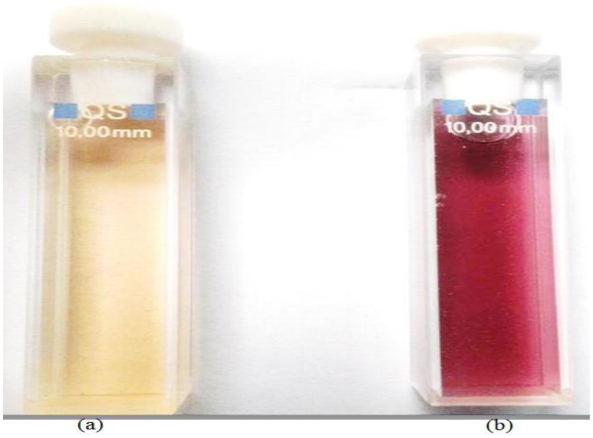

Figure 3.2. The color of **a)**Fe(III)-EDTA-NH_3 and **b)** Fe(III)-EDTA-NH_3-H_2O_2 complex.

The peroxocomplex is not stable and the color completely disappeared in 20 min (Figure 3.3). Further experiments werecarried out at 525 nm by photometrically.Baseline correction was also made by using suitable blank solution in this experiment and also for further experiments.

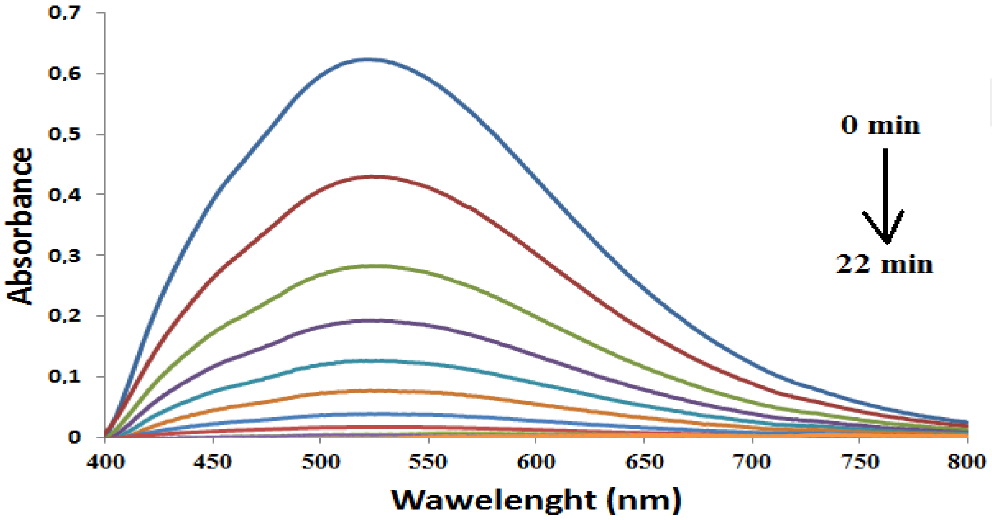

Figure 3.3. The absorbance change of Fe(III)-EDTA peroxo complex against to time Measurements were repeated after two minutes interval The concentrations of Fe(III),EDTA,NH_3,H_2O_2 are 0.003,0.05,5, $2x10^{-3}$mol/L respectively.

3.2. The Investigation of Stabilizator for Peroxo Complex

It was difficult to get reliable absorbance of peroxo complex because of the high decomposition rate. Therefore, the effects of varieties of compounds on decomposition rate were investigated for 3 minutes. The results are also presented in the absence and presence of stabilizator compounds between the Figures3.4 and 3.27.The decomposition rate was increased by the addition of $CuSO_4$ (Figure 3.6), $BiNO_3$(Figure 3.7), Fe(NO3)3 (Figure 3.12), $MnSO_4$(Figure 3.14), MoO_3(Figure 3.15), $K_2Cr_2O_7$ (Figure 3.17), KI (Figure 3.18, Na_2CO_3, $Na_2B_4O_7$ (Figure 3.24) (Figure 3.23), $AgNO_3$ (Figure 3.25) compounds. No effect was observed in the presence of $Na_2C_2O_4$(Figure 3.4), $HgCl_2$ (Figure 3.8), $(NH_4)_2Fe(SO_4)_2$ (Figure 3.5),SnO_2 (Figure 3.9), NaCl (Figure 3.13) and NaF(Figure 3.21).

Decomposition rate of the peroxo complex was slightly decrease by the addition of SnO_2 (Figure 3.9), $CoSO_4$ (Figure 3.10), $NiNO_3$ (Figure 3.16), KCl (Figure 3.19), K_2CrO_4 (Figure 3.20) Na_2SO_3 (Figure 3.22),. After addition of 4-buthylphenyl boronic acid (Figure 3.26) and $Na_2S_2O_3$ (Figure 3.27) to the medium decomposition rate of peroxo complex was decreased very high amount. Because of the low solubility of 4-buthylphenyl boronic acid in water, $Na_2S_2O_3$was selected as stabilizator reagent for further experiments.

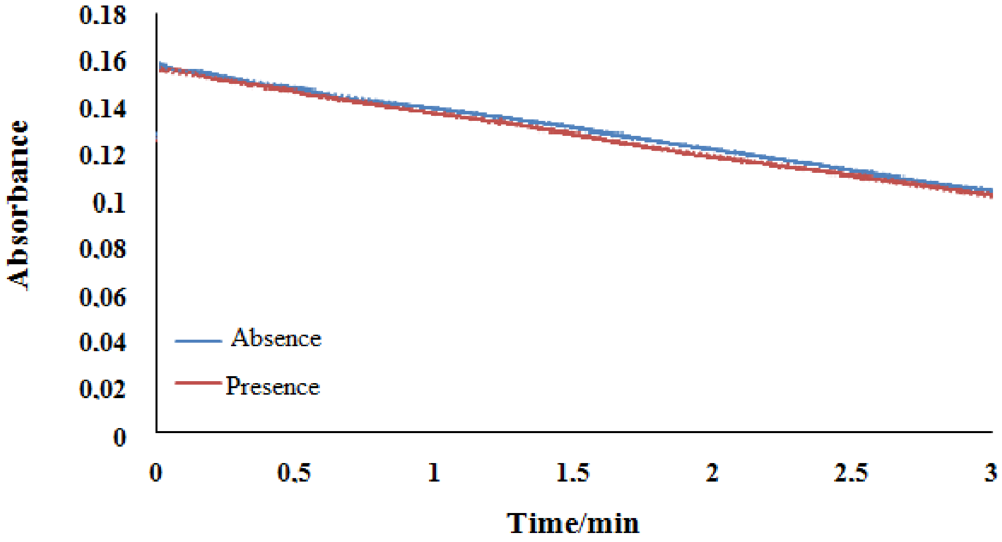

Figure 3.4. The decomposition rate of peroxo complex in the absence and presence of $Na_2C_2O_4$. (The concentration of Fe(III), EDTA, NH_3, $H_2O_2$and $Na_2C_2O_4$ are 0.002 ; 0.01; 0.5; 0.003; 0.005mol/L respectively)

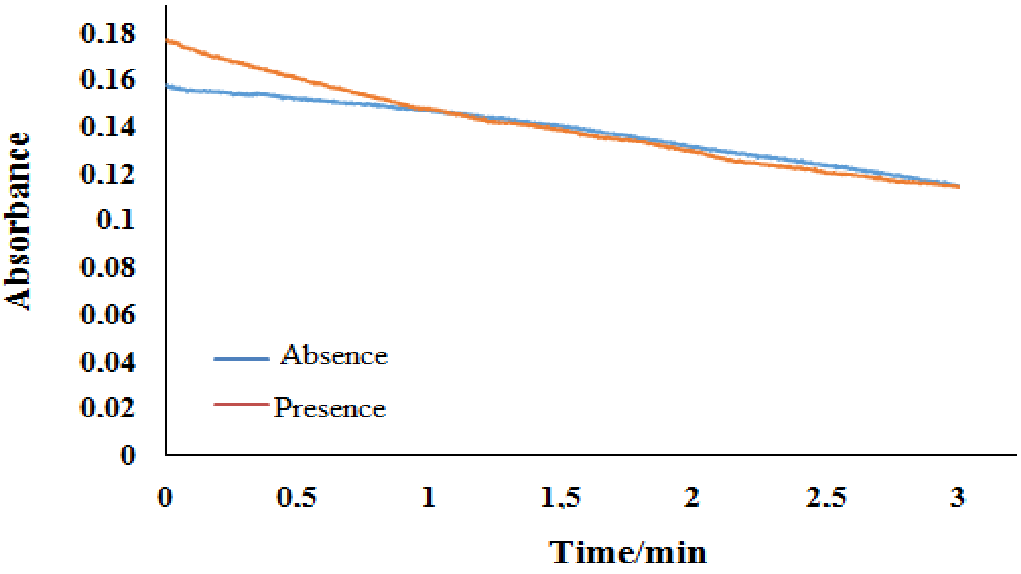

Figure 3.5. The decomposition rate of peroxo complex in the absence and presence of $(NH_4)_2Fe(SO_4)_2$. (The concentration of Fe(III), EDTA, NH_3, $H_2O_2$and $(NH_4)_2Fe(SO_4)_2$ are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L respectively)

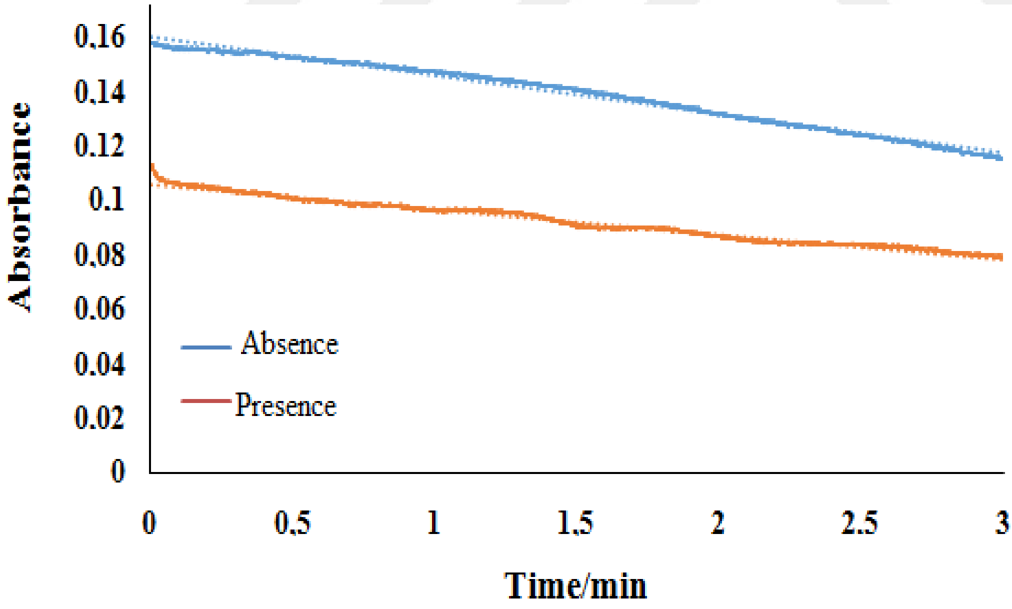

Figure 3.6. The decomposition rate of peroxo complex in the absence and presence of $CuSO_4$. (The concentration of Fe(III), EDTA, NH_3, $H_2O_2$and $CuSO_4$ are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L respectively)

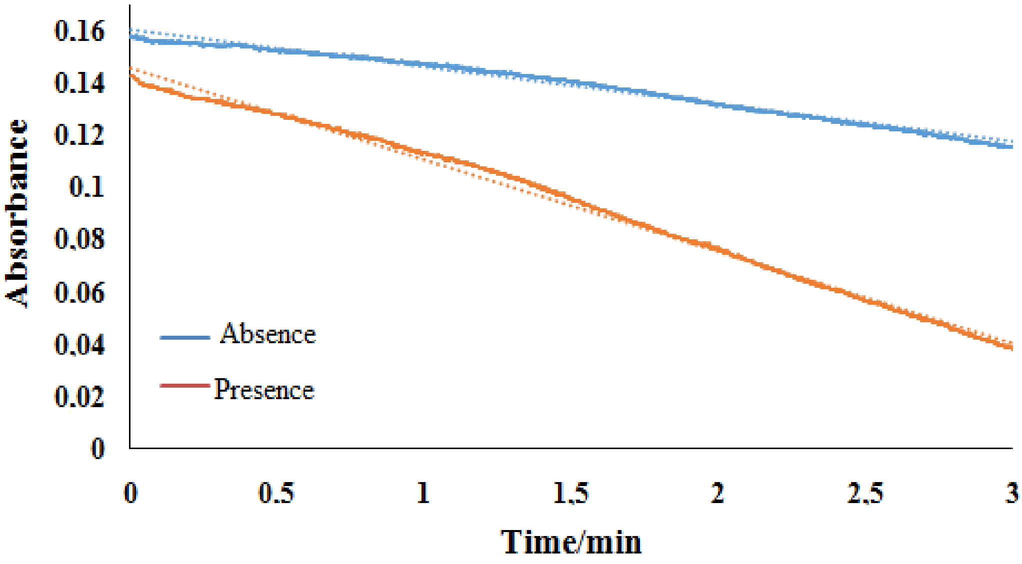

Figure 3.7. The decomposition rate of peroxo complex in the absence and presence of $Bi(NO_3)_3$ (The concentration of Fe(III), EDTA, NH_3, $H_2O_2$and $Bi(NO_3)_3$are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L respectively)

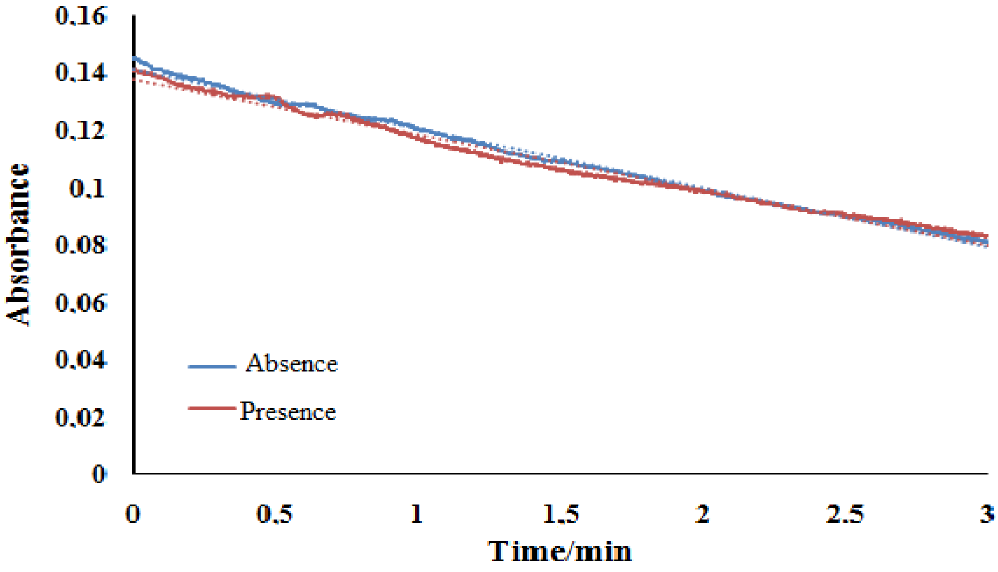

Figure 3.8. The decomposition rate of peroxo complex in the absence and presence of $HgCl_2$ (The concentration of Fe(III), EDTA, NH_3, $H_2O_2$and $HgCl_2$ are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L respectively)

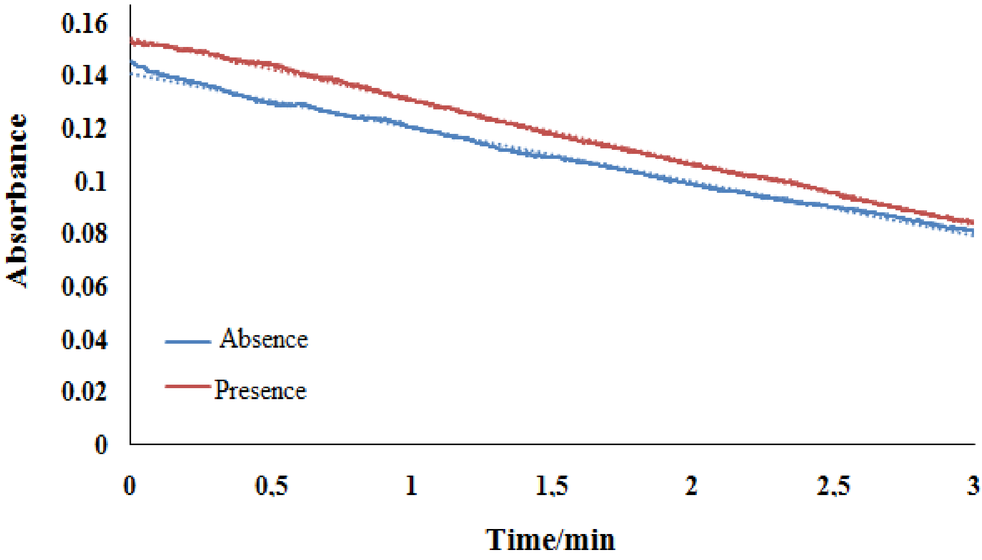

Figure 3.9. The decomposition rate of peroxo complex in the absence and presenceof SnO_2 (The concentration of Fe(III), EDTA, NH_3, $H_2O_2$and SnO_2 are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L respectively)

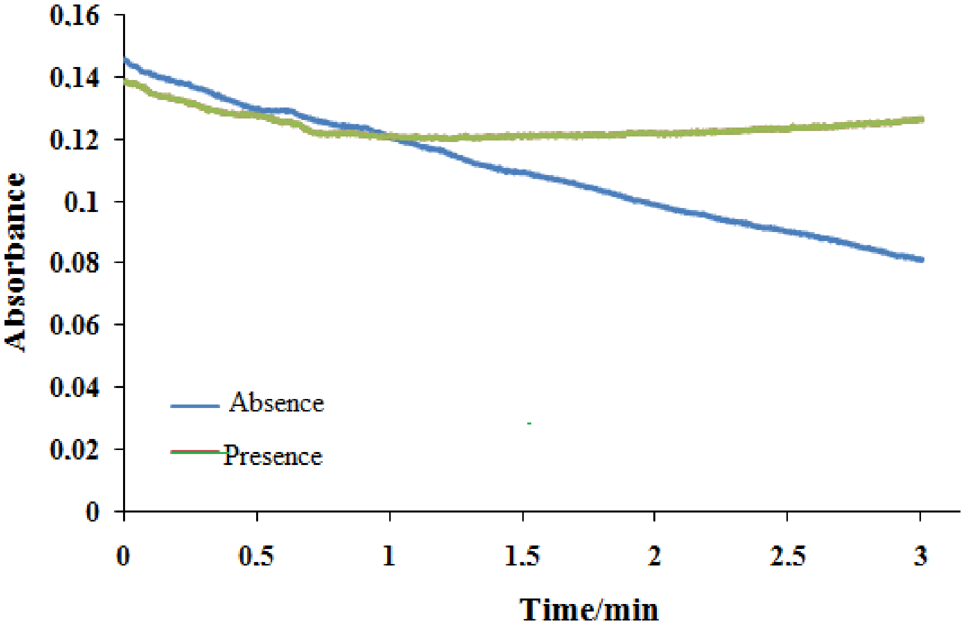

Figure 3.10. The decomposition rate of peroxo complex in the absence and presence of $CoSO_4$ (The concentration of Fe(III), EDTA, NH_3, $H_2O_2$and $CoSO_4$ are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L respectively)

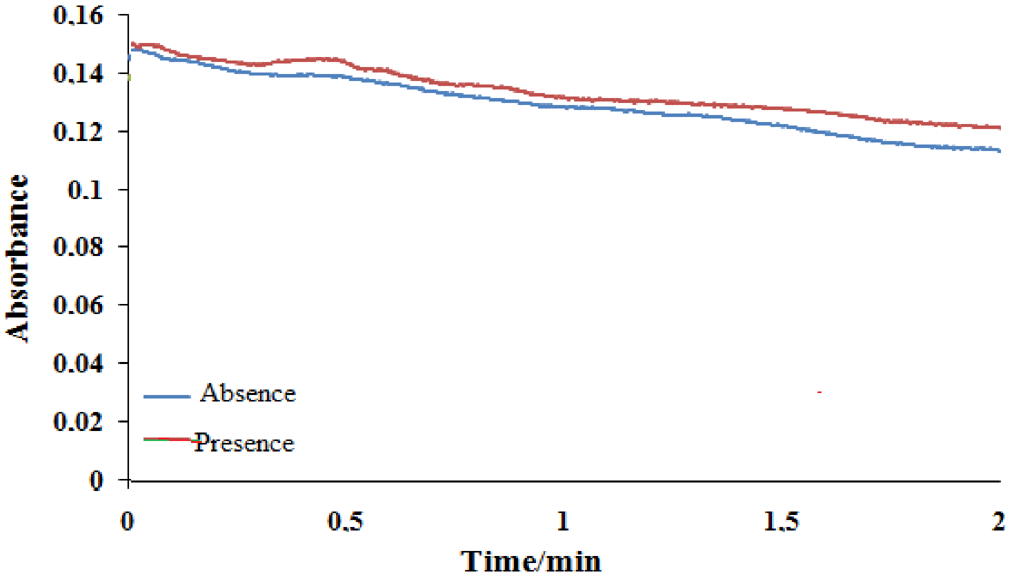

Figure 3.11. Thedecomposition rate of peroxocomplex in theabsenceand presence of $Pb(NO_3)_3$ (Theconcentration of Fe(III), EDTA, NH_3, $H_2O_2$and $Pb(NO_3)_3$are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L, respectively)

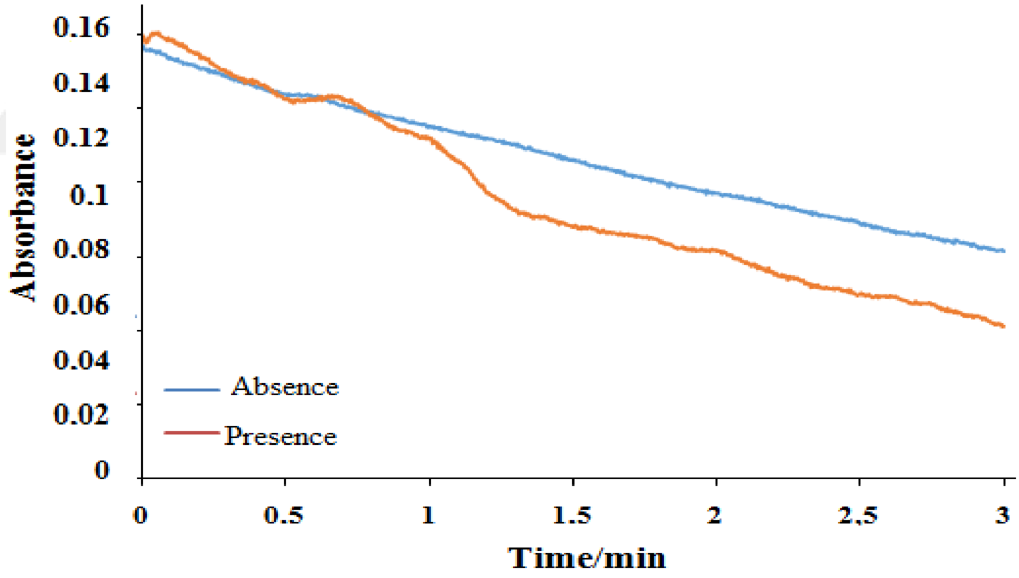

Figure 3.12. The decomposition rate of peroxo complex in the absence and presence of $Fe(NO_3)_3$(The concentration of Fe(III), EDTA, NH_3, H_2O_2andFe$(NO_3)_3$ are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L, respectively)

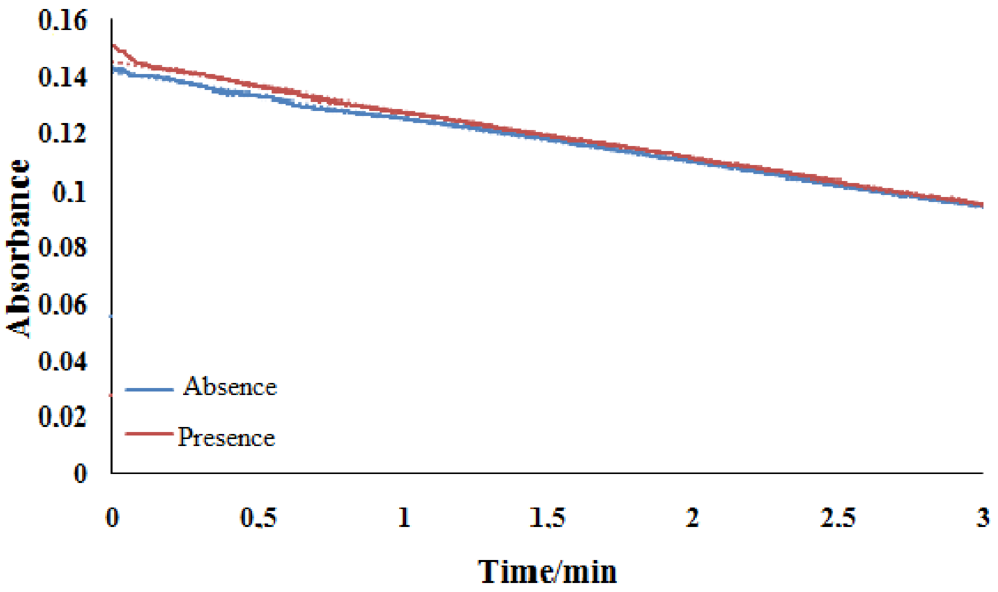

Figure 3.13. Thedecomposition rate of peroxocomplex in theabsenceand presence of NaCl(Theconcentration of Fe(III), EDTA, NH_3, H_2O_2andNaClare 0.002; 0.01; 0.5; 0.003; 0.005 mol/L, respectively)

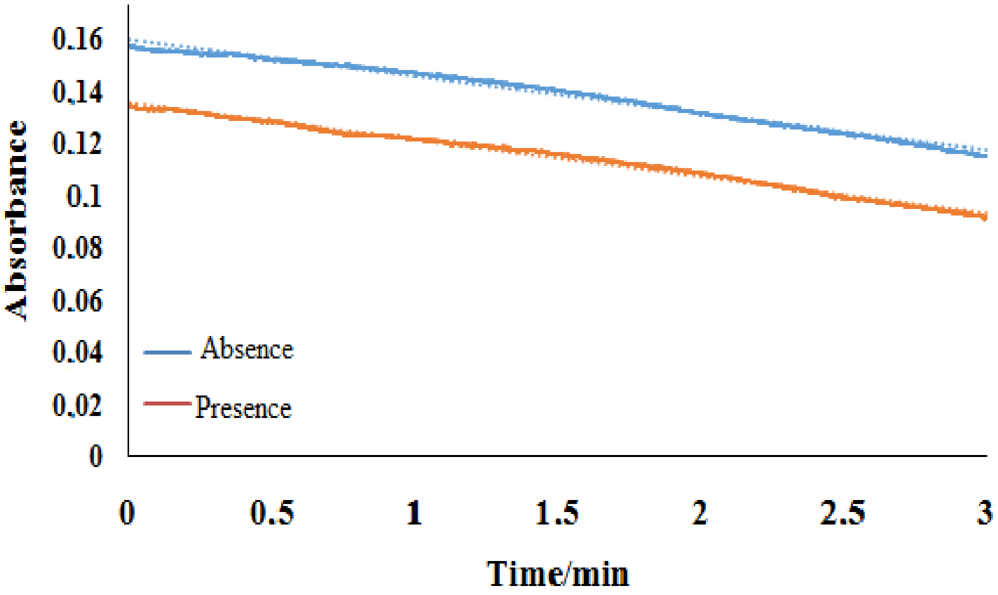

Figure 3.14. The decomposition rate of peroxo complex in the absence and presenceof$MnSO_4$(The concentration of Fe(III), EDTA, NH_3, $H_2O_2$and $MnSO_4$ are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L, respectively)

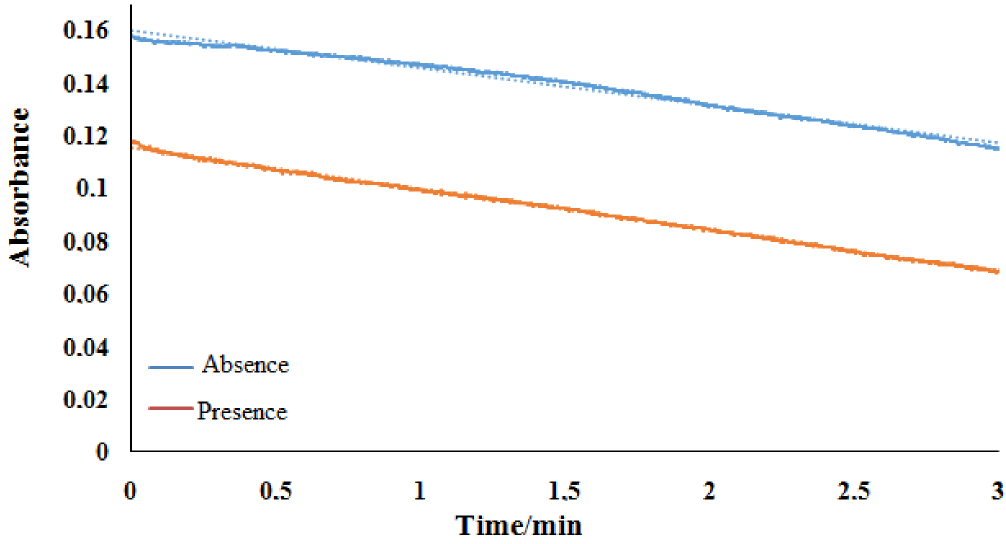

Figure 3.15. The decomposition rate of peroxo complex in the absence and presenceof MoO_3(The concentration of Fe(III), EDTA, NH_3, H_2O_2 and$MoO_3$are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L, respectively)

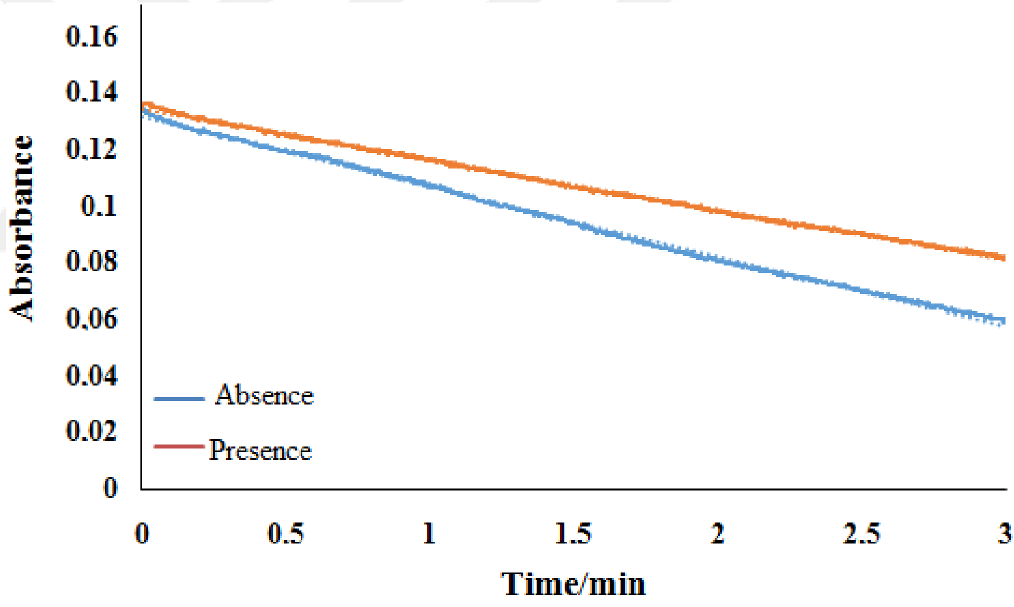

Figure 3.16. The decomposition rate of peroxo complex in the absence and presence of $Ni(NO_3)_2$ (The concentration of Fe(III), EDTA, NH_3, H_2O_2 and$Ni(NO_3)_2$ are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L, respectively)

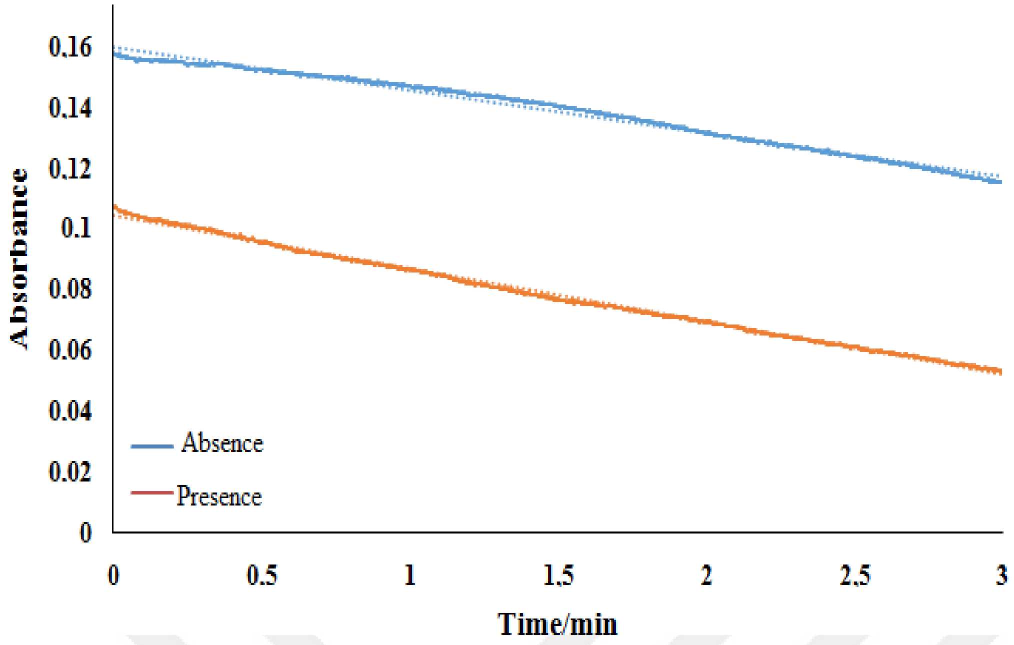

Figure 3.17. The decomposition rate of peroxo complex in the absence and precence of $K_2Cr_2O_7$ (The concentration of Fe(III), EDTA, NH_3, H_2O_2 and$K_2Cr_2O_7$are 0.002 ; 0.01; 0.5; 0.003; 0.005 mol/L, respectively)

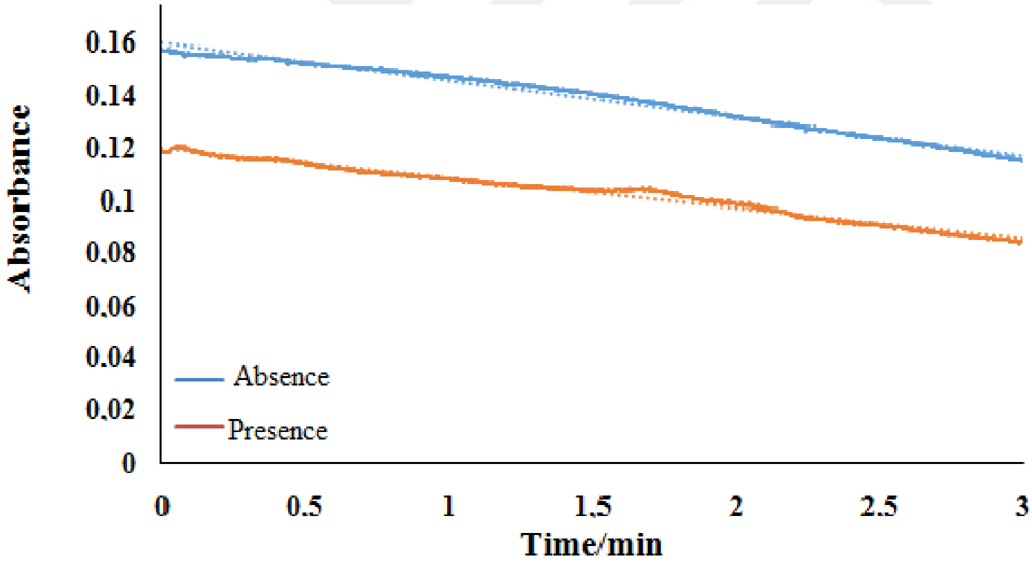

Figure 3.18. The decomposition rate of peroxo complex in the absence and presence of KI (The concentration of Fe(III), EDTA, NH_3, H_2O_2 and KI are 0.002 ;0.01; 0.5; 0.003; 0.005 mol/L, respectively)

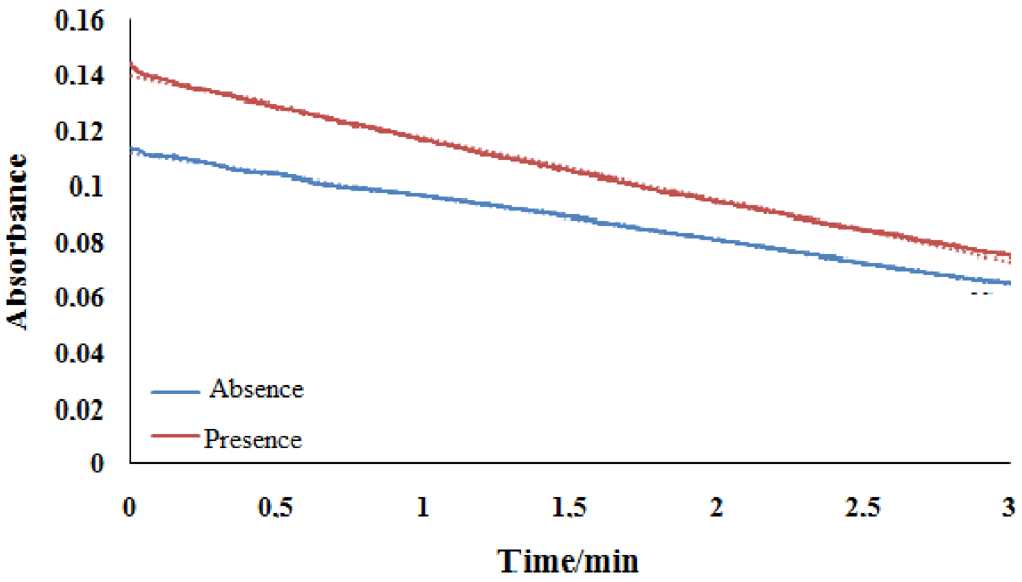

Figure 3.19. The decomposition rate of peroxo complex in the absence and presence of KCl (The concentration of Fe(III), EDTA, NH_3, H_2O_2 andKCl are 0.002 ;0.01; 0.5; 0.003; 0.005 mol/L, respectively)

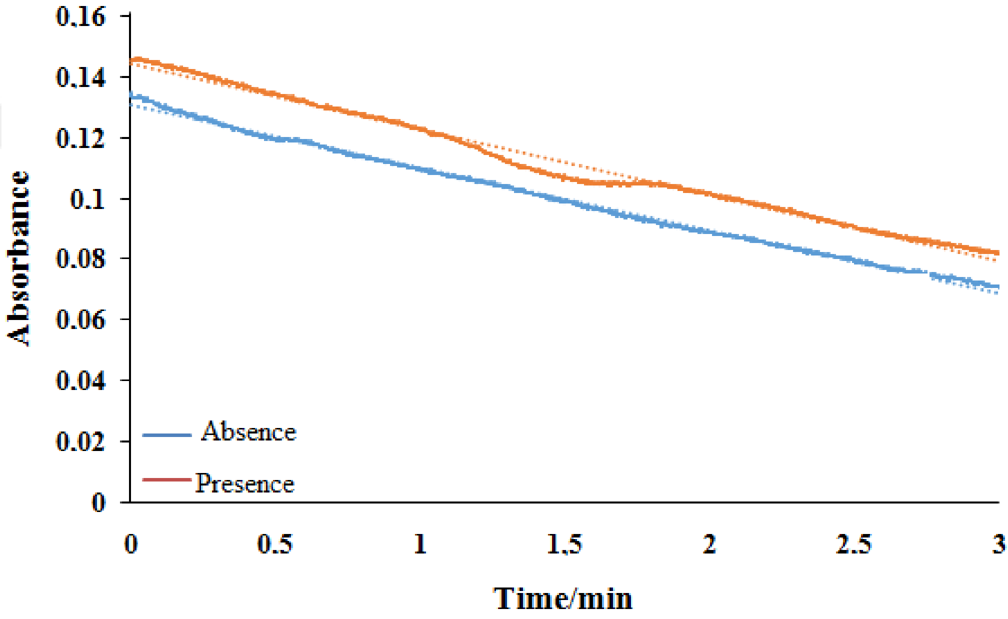

Figure 3.20. The decomposition rate of peroxo complex in the absence and presence of K_2CrO_4 (The concentration of Fe(III), EDTA, NH_3, H_2O_2 andK_2CrO_4 are 0.002 ;0.01; 0.5; 0.003; 0.005 mol/L, respectively)

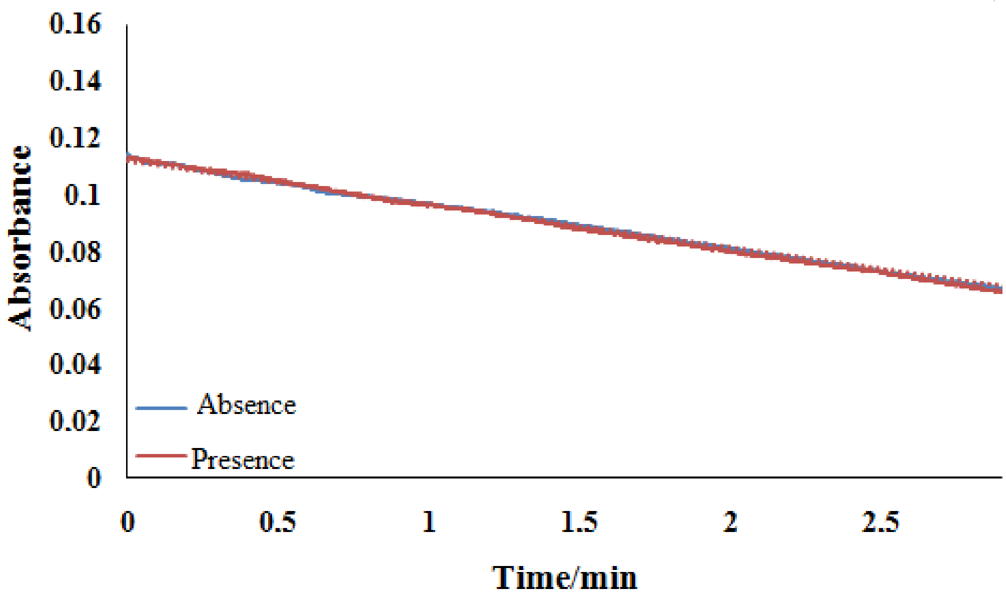

Figure 3.21. The decomposition rate of peroxo complex in the absence and presenceof NaF (The concentration of Fe(III), EDTA, NH_3, H_2O_2 andNaF are 0.002 ;0.01; 0.5; 0.003; 0.005 mol/L, respectively)

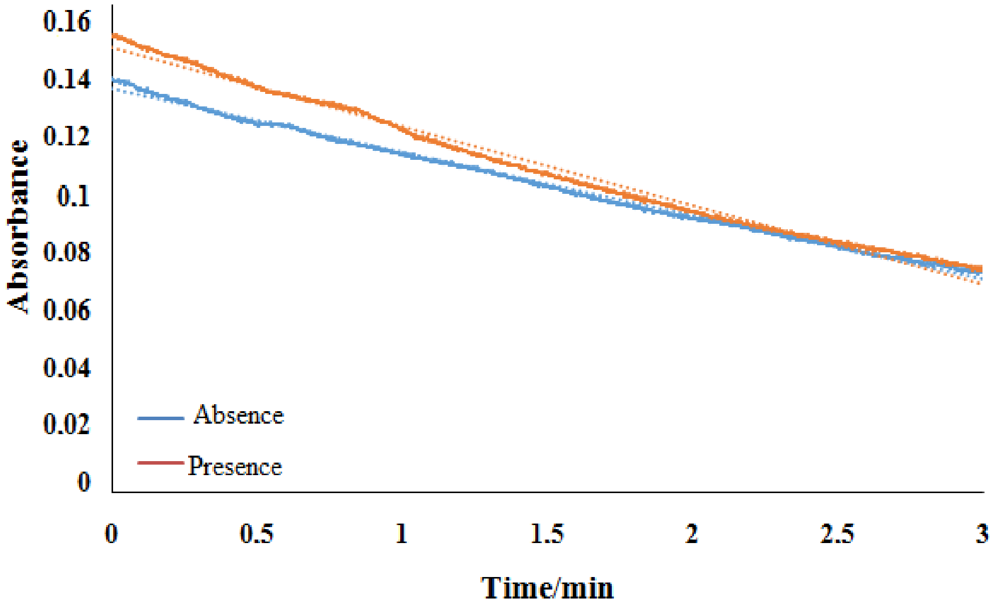

Figure 3.22. The decomposition rate of peroxo complex in the absence and presence of Na_2SO_3 (The concentration of Fe(III), EDTA, NH_3, H_2O_2 andNa_2SO_3 are 0.002 ;0.01; 0.5; 0.003; 0.005 mol/L, respectively)

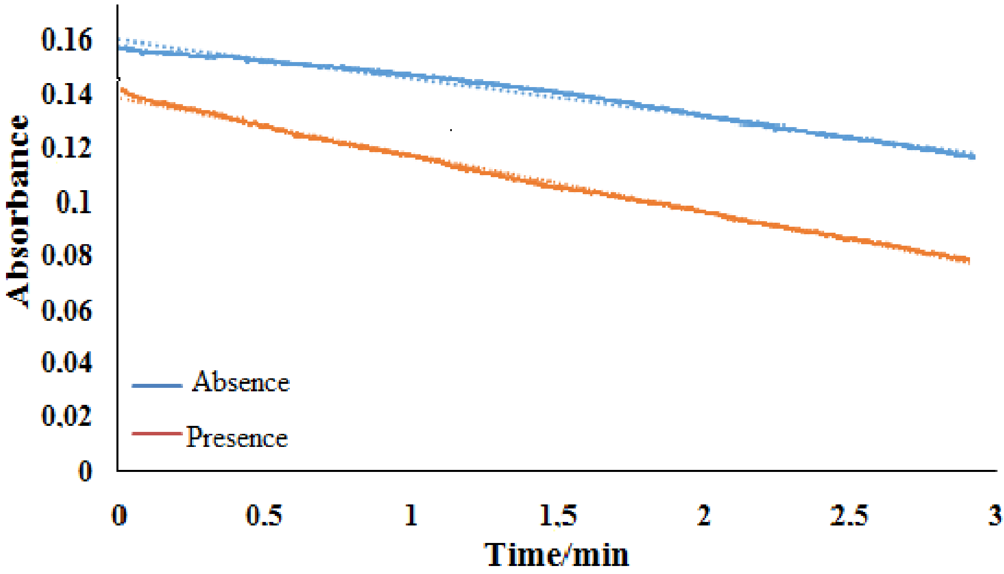

Figure 3.23. Thedecomposition rate of peroxocomplex in theabsenceandpresence of Na_2CO_3(Theconcentration of Fe(III), EDTA, NH_3, H_2O_2andNa_2CO_3 are 0.002 ;0.01; 0.5; 0.003; 0.005 mol/L, respectively)

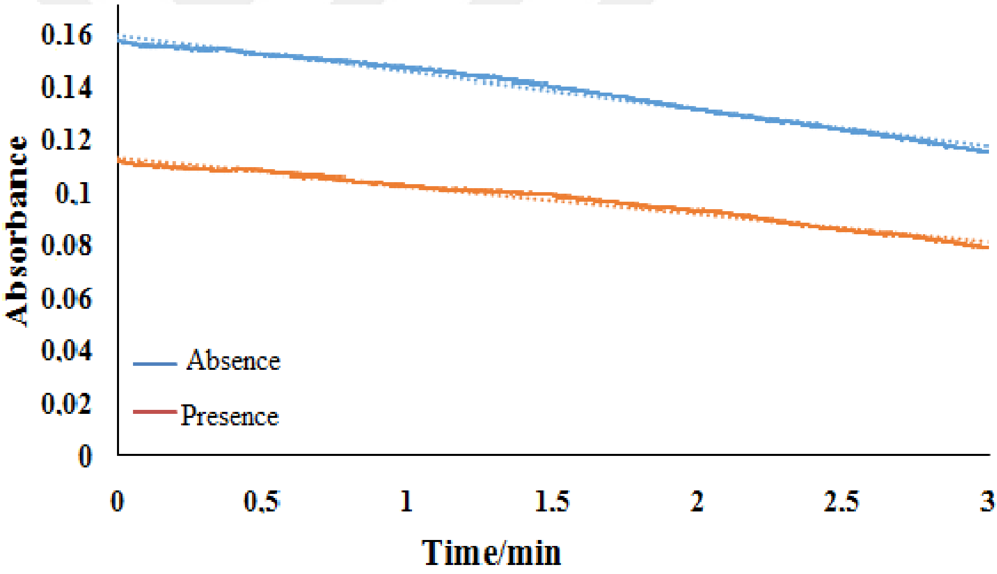

Figure 3.24. The decomposition rate of peroxo complex in the absence and presence of $Na_2B_4O_7$ (The concentration of Fe(III), EDTA, NH_3, H_2O_2 and$Na_2B_4O_7$ are 0.002 ;0.01; 0.5; 0.003; 0.005 mol/L, respectively)

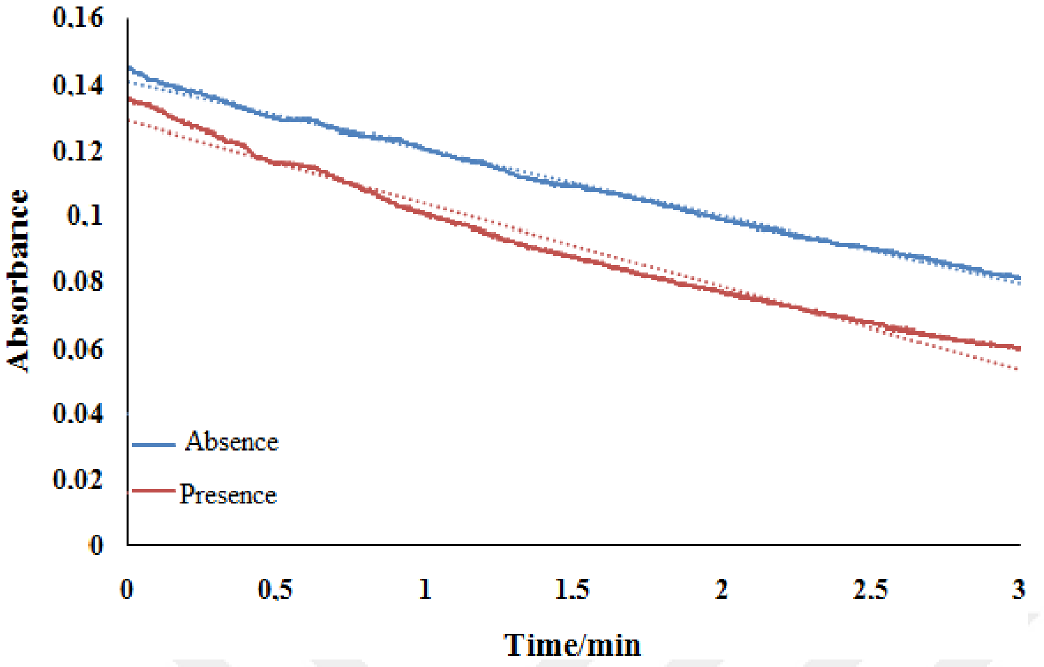

Figure 3.25. The decomposition rate of peroxo complex in the absence and presence of $AgNO_3$ (The concentration of Fe(III), EDTA, NH_3, H_2O_2 and$AgNO_3$ are 0.002 ;0.01; 0.5; 0.003; 0.005 mol/L, respectively)

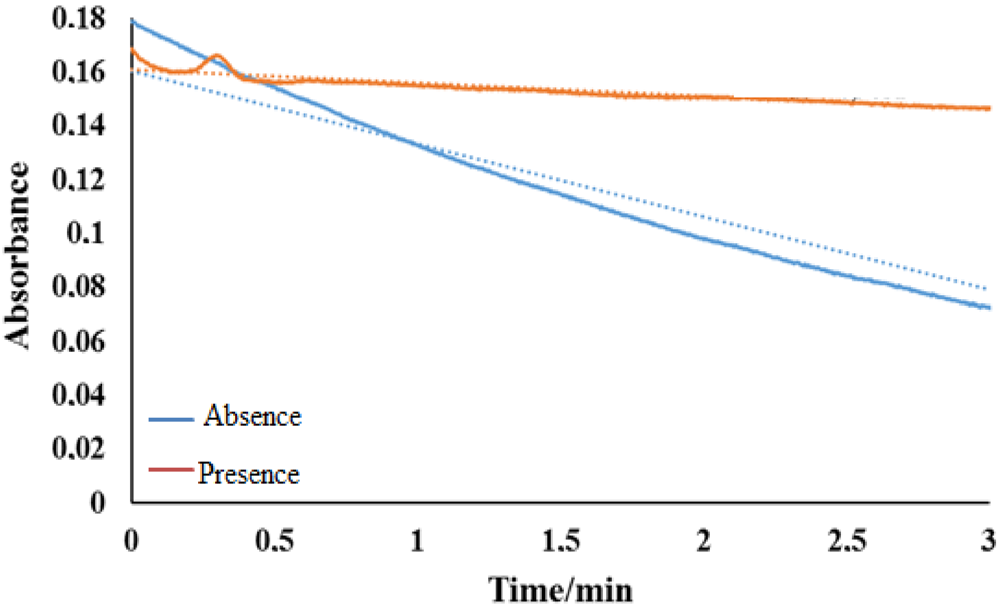

Figure 3.26. The decomposition rate of peroxo complex in the absence and precence of 4-buthylphenyl boronic acid (The concentration of Fe(III), EDTA, NH_3, H_2O_2 and4-buthylphenyl boronic acid are 0.002 ;0.01; 0.5; 0.003; 0.005 mol/L, respectively)

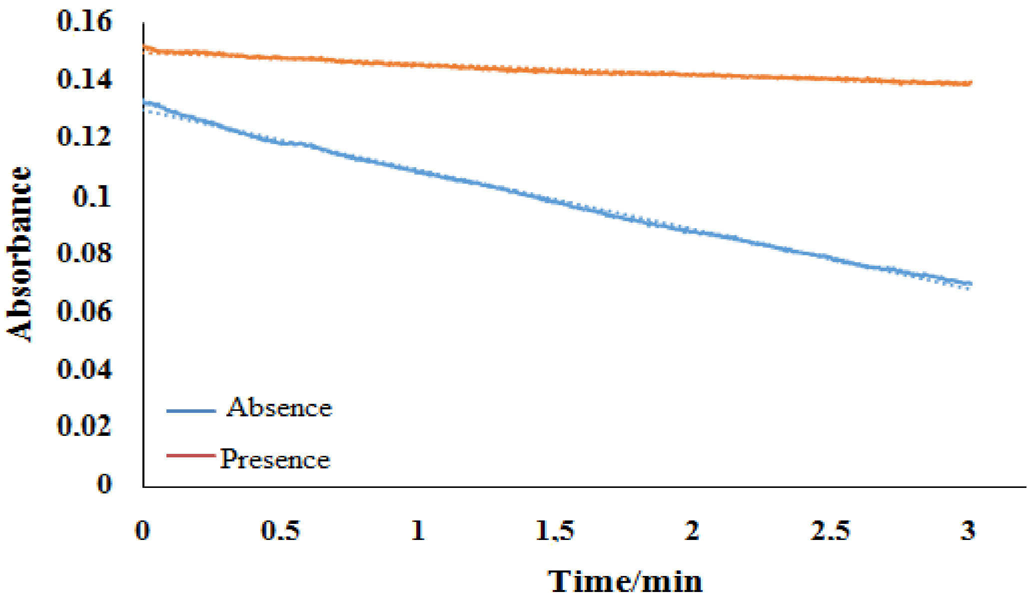

Figure 3.27. The decomposition rate of peroxo complex in the absence and precenceof $Na_2S_2O_3$ (The concentration of Fe(III), EDTA, NH_3, H_2O_2 and$Na_2S_2O_3$ are 0.002 ;0.01; 0.5; 0.003; 0.005 mol/L, respectively)

3.3. Optimization of the Proposed Method

3.3.1 Optimization of thiosulphate concentration

The optimization of the stabilizator concentration is important to get the more reliable results. The effect of $S_2O_3^{2-}$concentration was investigated between 0 and 0,08mol/L in the presence of peroxo complex (Figure 3.28). As can be seen from the figure,slight increase was observed by the addition of $S_2O_3^{2}$ until 0.04mol/L of concentration. The absorbance was not change in the concentration range of 0.04 mol/L and 0.06 mol/L of $S_2O_3^{2}$ concentration. Slight decrease was observed on the absorbance after 0.06 mol/L concentration of $S_2O_3^{2}$. Therefore, further experiments were carried out in the presence of 0.05 mol/L of $S_2O_3^{2}$ concentration.

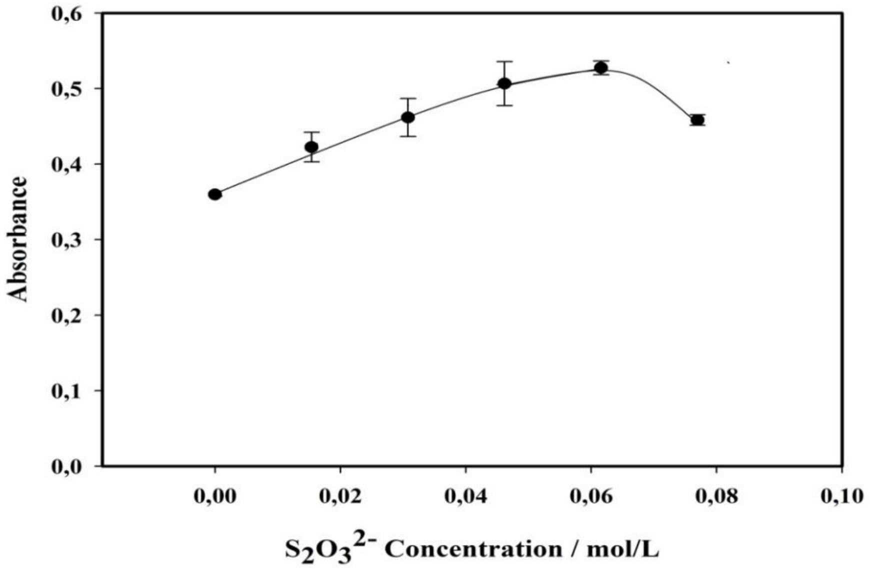

Figure 3.28: Optimization of of $S_2O_3^{2-}$concentration. The concentration of Fe(III), EDTA and NH_3, are 0.003, 0.05 and 5 mol/L, respectively.

3.3.2. Optimization of ammonia concentration

The effect of $NH_3$was investigated between 0.1 and 10 mol/L concentration range(Figure3.29).As can be seen from the Figure 3.29, absorbance of peroxo complex sharply increased by the addition of NH_3 until 5 mol/L concentration.A slight decreasewas observedat 10mol/L of NH_3 concentration. Thus, further experiments were carried out in the presence of 5mol/L of NH_3 concentration.

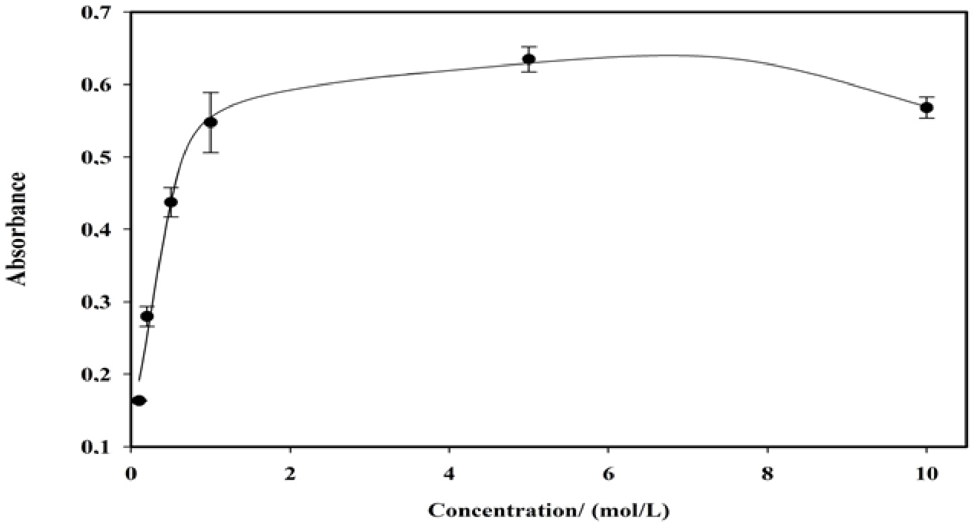

Figure 3.29. The effect of NH_3 concentration. Concentrations of Fe(III),EDTA and $S_2O_3$are $2x10^{-3}$, 0.01, and 0.05 mol/L, respectively**.**

3.3.3. Optimization of EDTA concentration

The effect EDTA concentration on the absorbance of peroxo complex was investigated between 0,004 and 0,08mol/L. As can be seen from the Figure 3.30,there was not a significant difference on the absorbance of the peroxo complexin the range of 0.004 and 0.05 mol/L concentration.After the concentration of EDTA exceeds 0.05 mol/L,a slight decrease was observed on the absorbance of peroxo complex. For this purpose, further experiments were carried out in the presence of 0.05 mol/L of EDTA concentration.

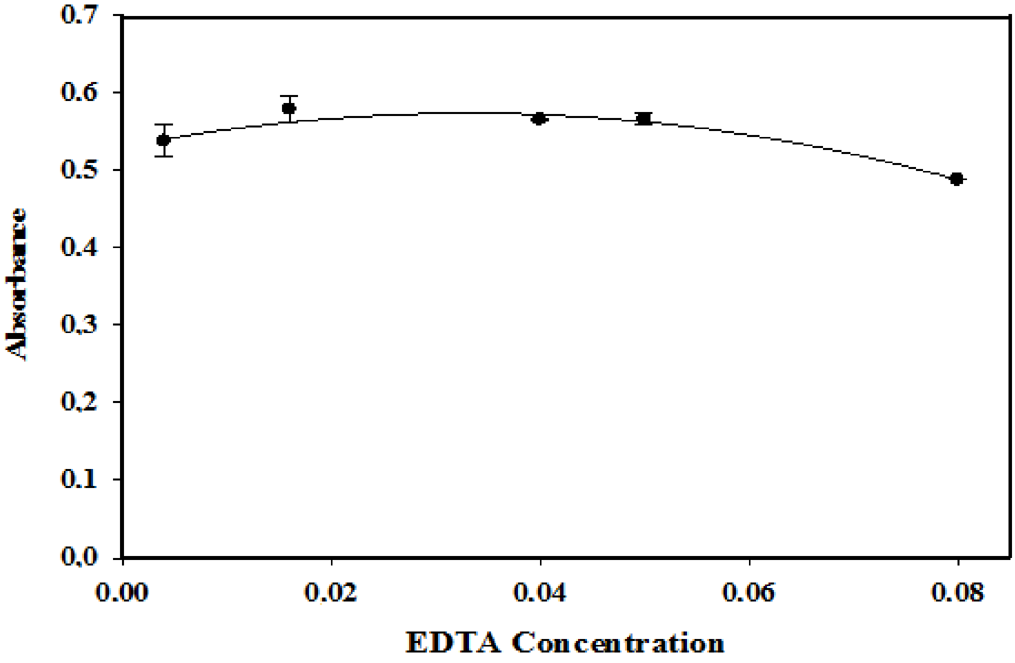

Figure 3.30. The effect of EDTA concentration.The concentrations of Fe(III),NH_3 and S_2O_3 were 0.002, 5, 0.05 mol/L, respectively.

3.3.4. Optimization of iron(III) concentration

Iron(III) catalyzes the decompose of H_2O_2 (Haber and Weiss, 1934) therefore, decomposition rate of peroxo complex is directly related to the Fe(III) concentration and it should be optimized. The effect Fe(III) concentration on the absorbance of peroxo complex was investigated between 0.0004 and 0.004 mol/L concentration range. Figure 3.31 shows that the absorbance of peroxo complex increased by the addition of Fe(III) until 0.002 mol/L of Fe(III) concentration. Then no important change was observed on the absorbance between 0.002 mol/L and 0.004 mol/L of Fe(III) concentration.

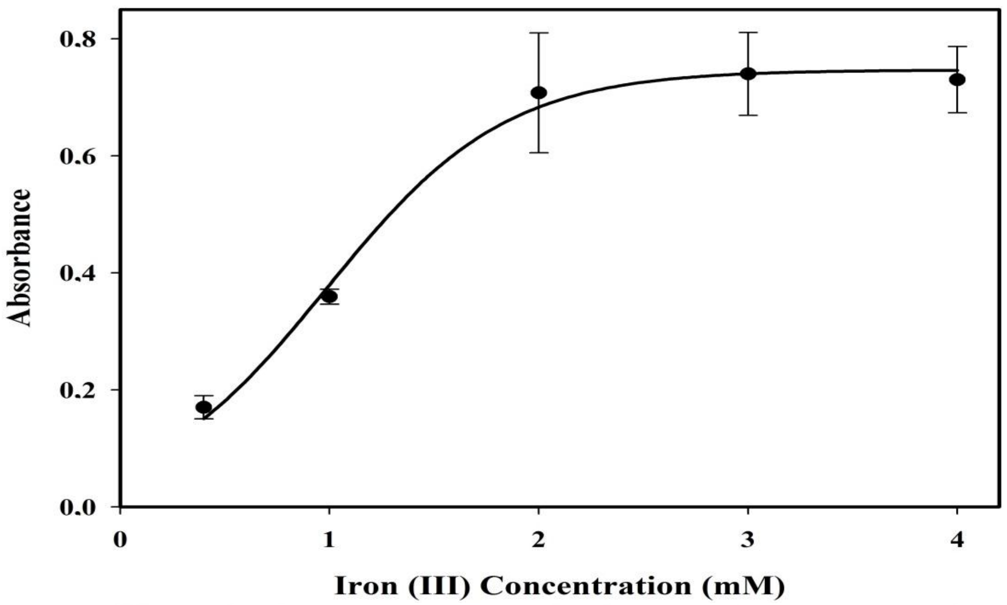

Figure 3.31. The effect of Fe(III) concentration on the absorbance

The decomposition rate of peroxo complex between 0.002 mol/L and 0.004 mol/L concentration of Fe(III) was also monitored for 2 minutes. As can be seen from the Table 3.1 the decomposition percentage of peroxo complex was not quite different for 30 seconds. However, 0.003 mol/L of Fe(III) showed the lower decomposition percentage for longer period.

Table.3.1. The change of decomposition percentage of peroxo complex by Fe(III) concentration against to time.

Time (s)	**Decomposition Percentage (%)**		
	2 mM Iron (III)	**3 mM Iron (III)**	**4 mM Iron (III)**
30	11.2	10.9	15.1
60	17.8	14.4	26.2
120	38.2	21.4	39.8

Therefore, optimum concentration of Fe(III) was selected as 0.003 mol/L.Optimum parameters were also summarized in Table 3.2.

Table.3.2. Optimum parameters

Parameters	**Condition**
Fe(III) concentration	0.003 mol/L
EDTA concentration	0.05 mol/L
$S_2O_3^{2-}$ concentration	0.05 mol/L
NH_3 concentration	5 mol/L
λ_{max}	525 nm

3.4. Selection of Measurement Time

Because of low stability of peroxo complex to identify the measurement, time is so important parameter to get the reliable results. Short measurement time increase the sensitivity but to make the measurement at the constant time is practically impossible. Therefore, the time zone should be selected from the low absorbance changing area of the peroxo complex.

For this purpose, the decomposition percentage of peroxo complex was monitored against the time under optimized conditions. The change of absorbance percentage of peroxo complex was compared in the absence and presence of $S_2O_3^{2-}$ (Table 3.3).Table 3.3 shows that the decomposition percentage of peroxo complex in the presence of $S_2O_3^{2}$ is about 5 times lower than according to the absence of $S_2O_3^{2}$.

Analysis times are generally complete in 30 seconds. The relative errors for completing the analysis at 15th second or 30th second are 2.2 % and 0.5 % for absence and presence of $S_2O_3^{2-}$ respectively. Therefore, the measurements were performed between 15 to 30 seconds.

Table 3.3.The change of decomposition percentage of peroxo complex against to time, in the presence and absence of $S_2O_3^{2-}$ under optimized conditions.

Time (s)	**Decomposition Percentage of Peroxo Complex (%)**	
	Absence	**Presence**
10	4.2	1.0
15	5.6	1.5
20	7.8	2.0
30	11.2	2.4
60	18.4	4.0
90	26.4	5.7
120	34.0	6.3

3.5. Selection of the Reagent Addition Order

The addition of reagent order effect the signal of colored complex and it should be optimized. For this purpose, addition orders of reagent were tested in the presence of 2.0 x10^{-3} M of H_2O_2 at the optimum reagent concentrations. As can be seen from the Table 3.4,the best results were obtained at the experiment 5. Therefore, a reagent addition order was used as well as experiment 5 for further experiments.

Table 3.4.The change of the absorbance of peroxo complex against to Reagent addition order in the presence and absence of $S_2O_3^{2}$ under optimized conditions

Experiment	**Reagent Addition Order**			**Absorbance**
	1	**2**	**3**	
1	Fe(III)-EDTA-NH_3	$S_2O_3^{2-}$	H_2O_2	0.515
2	$S_2O_3^{2-}$	H_2O_2	Fe(III)-EDTA-NH_3	0.385
3	$S_2O_3^{2-}$	Fe(III)-EDTA-NH_3-	H_2O_2	0.505
4	Fe(III)-EDTA-NH_3-$S_2O_3^{2-}$	H_2O_2		0.107
5	H_2O_2	NH_3	Fe(III)-EDTA-$S_2O_3^{2-}$	0.603

3.6. Analytical Merits of Proposed Method

The obedienceof absorbance values of peroxo complex against to H_2O_2 concentrationsto Beer's law was studied by varying the H_2O_2 concentration. A calibration curve was obtained by plotting absorbance of peroxo complex against H_2O_2 concentration in the range of $3.0x10^{-6}$ and $4.7x10^{-3}$mol/L (Figure 3.32).

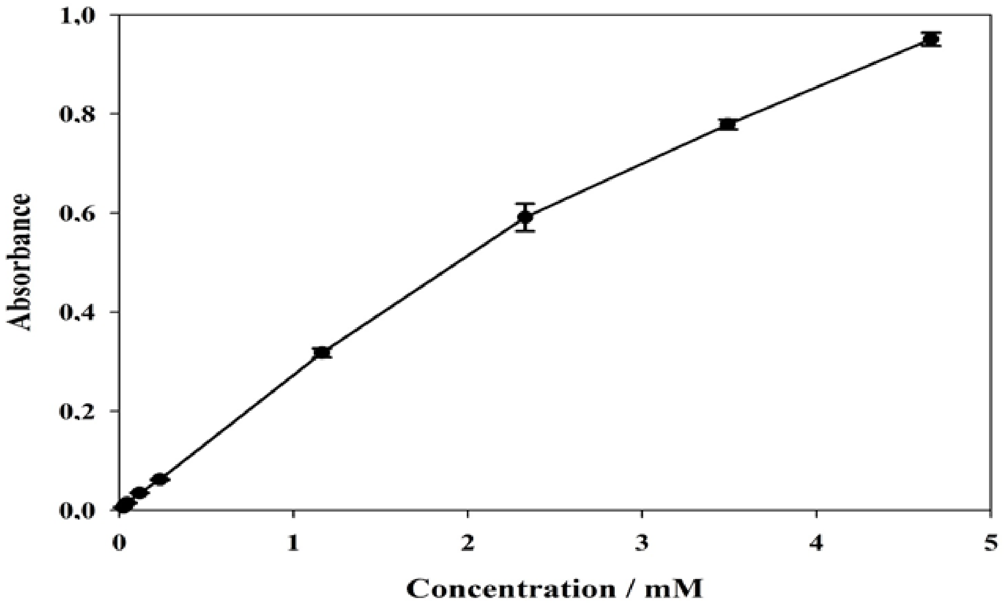

Figure 3.32.The absorbance change of peroxo complex against to H_2O_2 concentration under optimized conditions.

Good obedience to Beer’s law is obtained in the range of $3.6x10^{-6}$ and $4.08x10^{-3}$mol/L. Calibration curve for the determination $H_2O_2$is presented in the Figure 3.33. The increase in concentration of H_2O_2 shows a linear increase in the absorbance.

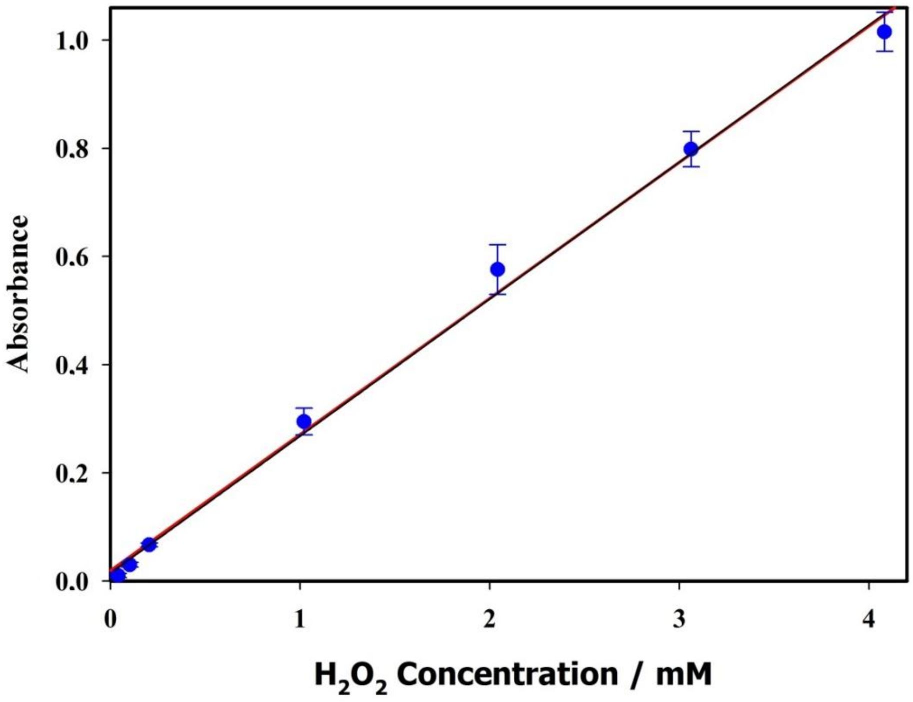

Figure 3.33. Calibration curve of the proposed method.

Molar absorptivity coefficient of peroxo complex was calculated as 267.36 L·mol^{-1}·cm^{-1} under optimized conditions.

"The Sandell's sensitivity is the concentration of the analyte (in µg mL^{-1}) which will give an absorbance of 0.001 in a cell of path length 1 cm and is expressed as µg cm^{-2}." (Sandell, 1939) Sandel's sensitivity was calculated from the following equation;

$S= \varepsilon s\ .y$

Where,

S= sandell's sensitivity

εs= specific extinction coefficient

y= concentration of the substance in mg/L

The LOD (limit of detection) and limit of LOQ (quantification) for the proposed method were calculated according to following equations:

LOD=3 x s/m

LOQ=10 x s/m

Where, s is the standard deviation of replicate measurement of blank signal under the optimized conditions and m slope of the calibration graph.

In order to evaluate the intraday and inter day precision of the proposed methods, a solution containing 2.0x10^{-4}mol/L concentration of $H_2O_2$was analyzedin five replicates during the same day and five consecutive days. The percentage of relative standard deviation(RSD%) were summarized in Table 3.5. The small values of the RSD% for intraday and interday indicate the high precision of the proposed methods. Analytical figure of merits such as the molar absorptivity coefficient, limit of detection and quantitation limit of the proposed method is also summarized in Table 3.5.

Table 3.5.Analytical figure of merits of the proposed method.

Parameters	**Value**
Calibration equation	y = 267.36x + 0.0093
Linearity	$5.0x10^{-6}$ and $4.08x10^{-3}$
R^2	0.9984
Molar absorptivity coefficient	$267 L\cdot mol^{-1}\cdot cm^{-1}$
Sandel's sensitivity	0.188 ($\mu g\ cm^{-2}$)
LOD	2.5×10^{-6} mol/L
LOQ	8.5×10^{-6} mol/L
Intraday RSD(for $2.0x10^{-4}$mol/L)	1.5 %
InterdayRSD (for $2.0x10^{-4}$mol/L)	6.1 %

The proposed method was compared with the other spectrophotometric method in Table 3.6 for the determination of H_2O_2 in real water samples. When we compare the proposed method with others, the compatible results were obtained.

.**Table 3.6.** Comparison of the proposed method with other spectrophotometric technique

Complexing Reagent	**λ_{max} (nm)**	**ε L $mol^{-1}cm^{-1}$**	**LOD µmol/L**	**Linear Range µmol/L**	**Water Type**	**Ref.**
Osmium (VIII) and m-carboxyphenylfluorone	580	5.21×10^4	Not given	59-12,000	Not applied	Hoshino et al, 2014
Toluidine blue	628	1.82×10^4	1.41	0.2-14	Rain Water	Sunill et al, 2008
p-hydroxyphenylacetic acid (PHPA)	432	Not given	290	1.47-1470	Rain Water	Tanner and Wolg, 1998
1,2-Di-(4-pyridyl)ethylene	442	$3.65x10^4$	Not given	8.82-441	Rain Water	Hauser and Kolar, 1968
horseradish peroxidase	420	Not given	0.1	0.06 – 2.7	Not applied	Kátia et al, 2005
Eriochrome black T	615	Not given	Not given	0.2-10	Not applied	Zhu et al, 1997
leuco crystal violet (LCV)	592	Not given	0.02	9.12-144	Marine water	Zhang et.al, 1994
N,N-diethyl.p-phenylendiamine (DPD)	320	Not given	1.7	125-1000	Surface, tap water	R. Schick et al., 1997
Fe(III)-EDTA	525	267	2.5	5-4080	Drinking, Tap, Sea water	Proposed Method

3.7. Interference studies

The effect of variety of well-known ions that are presented in the real water samples were studied for the interference studies. The effect of interfering ions in the determination of 1.0 × 10^{-4}mol/Lof H_2O_2 was studied. The effects of interfering ions were studied until 5.0x10^{-3}mol/Lconcentration. An error of ±5% in the reading of absorbance was considered tolerable concentration for H_2O_2 determination. The tolerance limits for various ions which are common in real waters are summarized in Table 3.7.

Table3.7.The list of interfering ions. (Concentration of $H_2O_2$and interfering ions were 1.0×10^{4}mol/L and 5.0×10^{-3}mol/L respectively)

Cations					
Interfering Ion	**Tolerance Limit (M)**	**Interfering Ion**	**Tolerance Limit (M)**	**Interfering Ion**	**Tolerance Limit (M)**
NH_4^+	No Int.*	Cu^{2+}	No Int.*	Bi^{3+}	No Int.*
Na^+	No Int.	Mn^{2+}	No Int.	Cr^{3+}	No Int.
K^+	No Int.	Pb^{2+}	No Int.	Fe^{2+}	1.0 x10^{-4}
Ag^+	No Int.	Zn^{2+}	No Int.	As^{3+}	No Int.
Mg^{2+}	No Int.	Co^{2+}	No Int.	Sn^{4+}	No Int.
Ca^{2+}	No Int.	Ni^{2+}	No Int.	Mo^{6+}	1.0x10^{-3}
Anions					
Interfering Ion	**Tolerance Limit (M)**	**Interfering Ion**	**Tolerance Limit (M)**	**Interfering Ion**	**Tolerance Limit (M)**
F^-	No Int.	NO_3^-	No Int	MoO_4^{2-}	No Int
Cl^-	No Int	NO_2^-	No Int	CO_3^{2-}	No Int
Br^-	No Int	SO_4^{2-}	No Int	CrO_4^-	No Int
I^-	No Int	$C_2O_4^{2-}$	No Int	SO_4^{2-}	No Int.

***No Int:**No interference

As can be seen from the Table 3.7 only Fe(II) shows serious interfering effect on H_2O_2 determination. The interfering concentration for Fe(II) was found as 1.0x10^{-4} mol/L. This concentration is lower than the maximum iron concentration of real water samples. Therefore, this method can be applicable for H_2O_2 determination in drinking water samples without any further sample preparation step.

3.8. Application of the Proposed Method to the Real Sample

The proposed method successfully applied to real water samples namely drinking water, tap water and seawater samples. The precision of the proposed method is evaluated by the three replicate analysis of water samples containing at three different concentrations H_2O_2 and are presented in Table3.7. Two different commercial bottled waters (A and B) were analyzed by proposed method. The H_2O_2 concentrations of all samples were blow the LOD value.

Table.3.8.The recovery values of the sample applications

Concentration (M)	**Recovery (%)**				
	Found	Bottled Water A	Bottled Water B	Sea Water	Tap Water
1.0x10^{-5}	<LOD	112±5	118±7	107±3	105±6
5.0x10^{-5}	<LOD	97±	91±8	90±5	107±8
1.0x10^{-4}	<LOD	100±4	102±5	102±4	98±7

4. GENERAL DISCUSSION AND CONCLUSION

In conclusion, we have developed a spectrophotometric method for the determination of H_2O_2 by using colored peroxo-iron(III)-EDTA complex in basic solutions. Stability of peroxo-Fe(III)-EDTA was not good enough for the reliable determination of $H_2O_2$and stability of peroxo complex was enhanced by adding $S_2O_3^{2-}$to medium as a stabilizator reagent. Developed method provided a sensitive, simple, rapid and inexpensive method for the determination of H_2O_2 in aqueous sample. Developed method allowed detection of H_2O_2 in a range from $5.0x10^{-6}$ and $4.08x10^{-3}$mol/L with high repeatability (%R.S.D: 1.6% for intraday, 6.5% for interday). The colored complex formation between H_2O_2 and Fe(III)-EDTA is very sensitive and can be applied for the determination of H_2O_2 in real water samples without any further sample preparation step. The LOD value of the proposed method is good enough for the determination of H_2O_2 in real water samples with acceptable recovery values. This method can be applied to other aqueous samples such as lake, river, well and mineral water samples.

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REFERENCES

"Hydrogen Peroxide Technical Library"(PDF). Retrieved 3 March2016.

Abbas, M.E., Luo, W., Zhu, Lihua., Zou, J.and Tang, H., 2010 ,Food Chemistry, 120 , 327-331 pp.

*Abrahams, S. C.; Collin, R. L.; Lipscomb, W. N. (1 January 1951). "The crystal structure of hydrogenperoxideActa Crystallographica**4**(1):15–20.doi:10.1107/S0365110X51000039*

Alamiry,M.A.H.,Harriman,A.,Mallon,L.J.,Ulrich,G.,Ziessel,R.,2008.Eur.J.Org. Chem. 2008(16),2774–2782.

Andreae, W., 1955, A Sensitive Method For The Estimation Of Hydrogen Peroxide İn Biological Materials. Nature 175, 859–860

Ansarı, I. A. and Datta A.K.,2009,An Overvıew Of Sterılızatıon Methods For Packagıng Materıals Used In Aseptıc Packagıng Systems Md.

Bader, H.; Sturzenegger, V.; Hoigne, J. 1988, Photometric Method For The Determination Of Low Concentrations Of Hydrogen Peroxide By The Peroxidase Catalyzed Oxidation of N-N diethyl-p-phenylenediamine. Water Res. 22, 1109–1115

Barbusıńskı*, K., 2009,Fenton Reactıon ,Controversy Concernıng The ChemıstryVol. 16, No. 3

Block,S. S.,(1991),Peroxygen compounds. İn Disinfection, sterilization, and preservation, (Lea &Febiger,Philadelphia, Pa),4th ed.pp167–181.

*Brauer, G, ed. (1963).Handbook of preparative inorganic chemistry*1. *Translation editing by Reed F. (2nd ed.). New York, N.Y.: Academic Press. p.140.ISB978-0-12-126601-1*

Brausam A., Eldik, R. van, .2004, *Inorg. Chem. 43*, 5351–5359

REFERENCES(CONTINIUE)

Brausam.A, 2009,Activation Of Hydrogen Peroxide By Heme And Non-Heme İron(III) Complexes

Burdo, T.; Seitz, W. 1975, Mechanisms of cobalt catalysis of luminal chemiluminescence. Anal. Chem. 47, 1639.

Canadian Centre for Occupational Health and Safety (CCOHS), 1998,*Cheminfo: Hydrogen*

Canadian Centre for Occupational Health and Safety (CCOHS),1998 *Cheminfo: HydrogenPeroxide solutions 35% and greater. Record number 198.*

Cho, J., Jeon, S., Wilson ,S.A., Liu,L. V., Kang,E. A., Braymer J. J., Mi Hee L., Hedman, B., . Hodgson K. O, Valentine,J., Selverstone ,E. I.,2011, Structure And Reactivity Of A Mononuclear Non-Haem İron(III)–Peroxo Complex, Nature, 478, 502–505 doi:10.1038/nature10535

Desesso, J.M., Lavin, A.L., Hsia, S.M. and Mavis, R.D., 2000, Food and Chemical Toxicology, 38: 1021-1041 pp.

Dougherty, Dennis A. And Anslyn, Eric V. (2005).*Modern Physical Organic Chemistry*,. University Science. p.122.ISBN1-89138931-9

Douglass, W C., Hydrogen Peroxide: Medical Miracle, 1992, Second Opinion Pub Inc.

Effkemann, S., Pinkernell, U, Karst, U., 1998, Analytica Chimica Acta , 363: 97-103 pp.

Falagas, M.E.; Thomaidis, P.C.; Kotsantis, I.K.; Sgouros, K.; Samonis,G. Karageorgopoulos, D.E. (July 2011). "Airborne hydrogen peroxide for disinfection of the hospital environment and infection control: a systematic review". Journal of Hospital Infection78(3): 171–177.doi:10.1016

REFERENCES(CONTINIUE)

Finlayson, B., Pitts,J., Pitts, J.N., 1986, Fundamentals and Atmospheric Techniques, Atmospheric Chemistry,Wiley, New York, 668-687 pp.

Glaze, W.H., Kang, J. and Chapin, D.H., 1987, Ozone, Science & Engineering: The Journal of the International Ozone Association Volume 9(Issue 4)

Gordon, G.; Cooper, W. J.; Rice, R. G. , 1992; Pacey, G. E. Disinfectant Residual Measurement Methods, 2nd ed. AwwaRF: Denver, CO; pp 429–445

Haber ,F.and Weiss ,J.,1934,The Decomposition of Hydrogen Peroxide by Iron Salts,

Hill, C. N. (2001).A Vertical Empire: The History of the UK Rocket and Space Programme, 1950–1971. Imperial College Press.ISBN978-1-86094-268-6

Hochanadel, C., 1952, Effects of cobalt γ-radiation on water and aqueous solutions. J. Phys. Chem 56, 587–594

Hoshino, M., Shinichiro Kamino, Mitsunobu Doi, Shingo Takada, Shota Mitani, Rika Yanagihara, Mamiko Asano, Takako Yamaguchi, Yoshikazu Fujita, Spectrochimica Acta Part A: Molecular and Biomolecular SpectroscopyVolume 117, 3 January 2014, Pages 814–816

Hunt, *Robert H.; Leacock, Robert A.; Peters, C. Wilbur; Hecht, Karl T. (1965,)*"Internal-Rotation in Hydrogen Peroxide: The Far-Infrared Spectrum and the Determination of the Hindering Potential"*(PDF).The Journal of Chemical Physics**42**(6): 1931.Bibcode:1965JChPh..42.1931H.doi:10.1063/1.1696228.*

Hydrogen Peroxide (H_2O_2),Menaging Hazardous Materiels Incidents, http://www.atsdr.cdc.gov/mhmi/mmg174

REFERENCES(CONTINIUE)

International Agency for the Research on Cancer (IARC), 1999, *Hydrogen Peroxide. Vol:71*, in *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.IARC: Lyon.

International Programme on Chemical Safety (IPCS), 1998, *Hydrogen Peroxide. PoisonsInformation Monograph. PIM 946*. WHO: Geneva.

International Programme on Chemical Safety (IPCS), 2000, *Hydrogen Peroxide (>60%) solution in water). International Chemical Safety Card: 0164*. WHO: Geneva.

Journal of Al-Nahrain University, 2013, Transition Metal Cations In Ammonium Alum-Urea Room Temperature Ionic Liquid].Vol.16 (3), September, pp.46-55, The Electronic Transition Behavior Cr (III), Fe (III), Fe (II) and Ni (II),

Karpelvel Leitner, N.; Dore, M., 1997, Mechanism of the reaction between hydroxyl radicals and glycolic, glyoxylic, acetic and oxalic acids in aqueous solution: consequence on hydrogen peroxide consumption in the H2O2/UV and O3/H2O2 systems. Water Res. 31,1383–1397.

Kieber, R.; Helz, G. 1986, Two-Method Verification Of Hydrogen Peroxide Determinations İn Natural Waters. Anal. Chem. 58, 2312–2315.

Klassem, N.; Marchington, D.; McGowan, H. 1994, H_2O_2 determination by I3− method and by KMnO4 titration. Anal. Chem. 66, 2921–2925.

Kok, G. L.; Thompson, K.; Lazrus, A.; McLaren, S. E. 1986, Derivatization Technique For The Determination Of Peroxides İn Precipitation. Anal. Chem. 58, 1192–1194

REFERENCES(CONTINIUE)

Kozan, J.V.B., Silva, R.P., Serrano, S.H.P., Lima, A.W.O., and Angnes, L., Analytica Chimica Acta, 2007, 591:200–207 pp

Lippert, A.R.,DeBittner,G.C.V.,Chang,C.J.,2011. Boronate oxidation as a bioorthogonal reaction approach for studying the chemistry ofhydrogen peroxide in living systems, Acc.Chem.Res.44(9),793–804.

Madsen, B.; Kromis, M. 1984, Flow injection and photometric determination of hydrogen peroxide in rainwater with N-ethyl-N-(sulfopropyl)aniline sodium salt. Anal. Chem. 56, 2849–2850.

Masschelein, W.; Denis, M.; Ledent, R., 1977, Spectrophotometric Determination Of Residual Hydrogen Peroxide. Water Sewage Works; August, Pp 69–72.

McDonnell,G.andDenver ,R.A.,Antiseptics and Disinfectants: Activity, Action, and Resistance,STERIS Corporation, St. Louis Operations, St. Louis, Missouri 63166,and Welsh School of Pharmacy, Cardiff University, Cardiff CF1 3XF, United Kingdom

Miller, E.W.,Dickinson,B.C.,Chang,C.J.,2010. Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling, Proc.Natl.Acad.Sci.USA107(36), 15681–15686.

Miller, W.; Kester, D. 1988., Hydrogen peroxide measurement in seawater by (p–hydroxyphenyl)acetic acid dimerization. Anal. Chem. 60, 2711–2715

Mottola, H.; Simpson, B.; Gorin, G. Absorptiometric determination of hydrogen peroxide in submicrogram amounts with leuco crystal violet and peroxidase as catalyst. Anal. Chem.1970, 42, 410

Munter ,R. 2001, Advanced Oxıdation Processes – Current Status And Prospects

REFERENCES(CONTINIUE)

Paulsen, C.E., Carroll. K.S., Orchestrating redox signaling networks through regulatory cysteine switches.ACS Chem. Biol.2010;5:47–62

Peroxide solutions of less than 8%. Record number 77.

Perschke, H.; Broda, E. Determination of very small amounts of hydrogen peroxide. Nature, 1961, 190, 257–258

Petrucci, Ralph H. (2007).General Chemistry: Principles & Modern Applications(9th ed.). Prentice Hall. p.606.*ISBN0-13-149330-2.*

Price, D.; Worsfold, P.; Mantoura, R. 1994, Determination Of Hydrogen Peroxide İn Sea Water By Flow İnjection Analysis With Chemiluminescence Detection. Anal. Chim. Acta 298,

Rhee, S.G.,1999. Redox signaling: hydrogen peroxide as intracellular Messenger Exp.Mol.Med.31(2),53–59.

Rhee, S.G.,2006. Cell signaling. H2O2, A Necessary Evil For Cell Signaling ,Science, 312,1882–1883.

Sandel, E. , 1939, Colorimetric Determinations of Trace Metals3rd ed. Interscience Publishers: New York, NY, p 929.

Schick, R.; Strasser, I.; Stabel, H. 1997, Fluorometric determination of low concentrations of H2O2 in water: comparison with two other methods and application to environmental samples and drinking water treatment. Water Res. 31, 1371–1378

Schumb, W.; Satterfield, C.; Wentworth, L. , 1955, Hydrogen Peroxide. Reinhold Publishers: NewYork, NY.

Scott, W. W. 1939,Scott's Standard Methods of Chemical Analysis, 5th ed. D. Van Nostrand Co.: New York, NY.

REFERENCES(CONTINIUE)

Sharma, V K., Szilágyi, P. Á., Homonnay,Z., KuzmannE. and Vértes., A,2005Mössbauer Investigation of Peroxo Species in the Iron(III)–EDTA–H_2O_2 System **,** DOI:10.1002/ejic.200500299

Shengmin, L., Jun ,S., Leslie Campbell-Palmer, 2009, Scientia Horticulturae, 120 : 336-341 pp.

Shepherd, S.*"Brushing Up on Gum Disease" FDA Consumer*. Archived fromthe originalon 14 May 2007. Retrieved7 July2007.

Solvay Chemical Inc, 2004a, Determination of Hydrogen Peroxide; Ceric Sulfate Method; TDS HH-5201; Brussels, Belgium,

Solvay Chemical Inc., 2004b.Determination of Hydrogen Peroxide Concentration; TDS HH-118;Brussels, Belgium,

Sunder, M.; Hempel, D., 1997, Oxidation of tri- and perchloroethene in aqueous solution with ozone and hydrogen peroxide in a tube reactor. Water Res. 31, 33–40.

SunilK., Narayana, B. Spectrophotometric Determination of Hydrogen Peroxide in Water and Cream Samples, Bulletin of Environmental Contamination and Toxicology, 2008, Volume 81, Issue 4, 422–426.

Tanner,P.A.,Wong, A.Y.S., (1998),Spectrophotometric Determination Of HydrogenPeroxide In RainwaterAnalytica Chimica Acta 370 279±287

Thacker, E., 2010,The Magic of Hydrogen Peroxide, Hartville.

US Peroxide. http://www.h2o2.com (accessed June 2015)

Wernimont, E. J, 2006,System Trade Parameter Comparison of Monopropellants: Hydrogen Peroxide vs Hydrazine and Others * General Kinetics Inc., Huntington Beach, CA 92649

REFERENCES(CONTINIUE)

Winterbourn, C.C.,2008. Reconciling the chemistry and biology of reactive oxygen species, Nat.Chem.Biol.4(5),278–286.

World Health Organisation (WHO), 1973, *Hydrogen Peroxide. 267. Joint FAO/WHO Expert Committee on Food Additives*, in *WHO food additives series No 5*. WHO:Geneva.

Worthington Biochemical Corporation. http//www.worthington-biochem.com/HPO/default.html (accessed Dec 2008).

Yamashiro, N.; Uchida, S.; Yamada, R. 2004, Determination of hydrogen peroxide in water by chemiluminescence detection. J. Nucl. Sci. Technol. 41, 890–897.

Yifei, Y.,Shaolin, M., 2005, Biosensors and Bioelectronics, 21, 74–78 pp

Yuan,L.,Lin,W.,Xie,Y.,Chen,B.,Zhu,S.,2012. Single fluorescent probe responds to H2O2, NO, and H2O2/NO with three different sets of fluorescence signals,J.Am.Chem.Soc.134(2),1305–1315.

Zhang L-S.,George T.F. Wong 1994,Spectrophotometric Determination Of H2O2 In Marine Waters With Leuco Crystal Violet, Talanta Volume 41, Issue 12 December, Pages 2137-2145

Zhu, M., Huang X., Liuzhan, L, Shen, H., Spectrophotometric determination of hydrogen peroxide by using the cleavage of Eriochrome black T in the presence of peroxidase, Talanta, Issue 8, 1997, Pages 1407–1412

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